EVALUATION OF *BRADYRHIZOBIUM* STRAINS FOR LUPINS IN FINNISH CONDITIONS

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Tiivistelmä—Referat— Abstract
Four different *Bradyrhizobium* sp. (lupin) inoculants were investigated in both greenhouse and field experiments to compare their effects on growth, yield and biological nitrogen fixation. Narrow-leafed and white lupin of the greenhouse experiment compared the strains test in different potting media in a controlled environment, while field experiment tested their performance in field conditions.

The greenhouse experiment was conducted with 3 narrow-leafed lupin cultivars (Haags Blaue, Boruta and Sonet) and 1 white lupin cultivar (Ludic). Plants were grown in 3 different potting media (soil, 2 peat : 1 sand and 1 peat : 2 sand) with 5 *Bradyrhizobium* treatments (uninoculated control, commercial peat inoculant of HAMBI 3118 and liquid cultures of HAMBI 3115, HAMBI 3116 and HAMBI 3118). Plants were grown in a greenhouse unit with average day and night temperature of 22°C and 18°C. Plants were illuminated by using cool white fluorescent tubes maintaining 18 hours day and 6 hours night.

In the greenhouse experiment, inoculation significantly increased shoot (117.1-141.9%), root (45.8-64.4%) and nodule (237.0-266.6%) dry weight, plant height (38.3-46%), nodule number (620-659%) and chlorophyll content (29.0-38.5%) over the values found in uninoculated controls. Soil type or potting medium also influenced lupin growth and yield, with better results observed in soil, poorer in 2 peat : 1 sand and poorest in 1 peat : 2 sand. Best performaces were obtaind by inoculating with HAMBI 3115 strain in soil. Uninoculated plants and even inoculated plants grown in peat-sand potting medium, showed relatively poor results, which was more obvious in high-yielding cultivars, Boruta and Ludic, than in low-yielding cultivars, Haags Blaue and Sonet. Inoculation treatments also showed significantly higher shoot (3.15-3.39% N) and root (1.96-2.54% N) nitrogen content. Biological nitrogen fixation rate, measured by the nitrogen difference method, ranged between 87.9 and 90.8% depending on both bacterial strain and host cultivar.

The field experiment showed significant increases in shoot (14.4-47.9%), root (11.9-29.1%) and seed (13.8-68.6%) dry weight, plant height (3.6-10.7%), pod plant^{-1} (10.7-50.6%) and chlorophyll content (5.7-20.7%) following inoculation of the three narrow-leafed lupin cultivars.

Uninoculated plants grown in soil in the greenhouse experiment and in the field experiment both produced some nodules, which showed the evidence of presence of indigenous nodule-forming and nitrogen-fixing bacteria. Among the 3 liquid cultures, HAMBI 3115 performed best in terms of lupin growth, yield and biological nitrogen fixation in both greenhouse and field experiments. The performance of the peat-based commercial inoculant of HAMBI 3118 strain exceeded all other inoculants in the field experiment but not in the greenhouse experiment, showing the importance of the carrier.

The results indicated that lupin growth and yield are strongly affected by *Bradyrhizobium* inoculation and soil characteristics. Selection of a suitable *Bradyrhizobium* strain for inoculation and growing cultivars according to their soil preferences can maximize lupin yield. The suitability of HAMI 3115 for making peat-based inoculants should be tested.

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Lupinus angustifolius, Lupinus albus, Bradyrhizobium, nitrogen content, biological nitrogen fixation.

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1 INTRODUCTION

Legumes are mainly recognised for their high protein and oil contents and widely used as an important source of food and feed (Duranti & Guis 1997). According to FAOstat data, during 2009 the world's pulse production was 62 million tons and was harvested from 71 million hectares (FAO 2012a). Each year grain legumes contribute 21 million tons of fixed nitrogen to agriculture (Herridge et al. 2008). Legumes are important not only for their nitrogen fixing capabilities but also for their ability to improve soil properties through nitrogen mineralization (Carpenter-Boggs et al. 2000).

Among the cultivated legumes, lupin is important as it contains high amounts of protein, fibre, oil and sugar (Erbas et al. 2005). At present, the most important lupin producing country worldwide is Australia (629,000 t), whereas in Europe, lupin is cultivated mostly in Poland (25,900 t), Russia (9,740 t), Italy (5,400 t), Spain (3,700 t), Greece (400 t) and Hungary (400 t) (FAO 2012b). Total production of the top 15 lupin producing countries during 2010 was 746,374 t (FAO 2012b). Of the cultivated lupins, *Lupinus angustifolius* L. as grown in Central and Eastern Europe can also be a potential crop for Finnish condition (Peltonen-Sainio et al. 2009, Stoddard et al. 2009).

The genus *Lupinus* has more than 400 species but only four of them are commercially cultivated and have agronomic interest: *L. angustifolius* (blue or narrow-leafed lupin), *L. albus* L. (white lupin), *L. luteus* L. (yellow lupin) and *L. mutabilis* Sweet (Andean lupin) (Reinhard et al. 2006). The first three originated in the Mediterranean area and are known as 'Old World' species while *L. mutabilis*, which originated in South America, is known as a 'New World' species (Hondelmann 1984).

Among the cultivated species, white lupin and narrow-leafed lupin have outstanding grain protein content and are particularly suitable for ruminant diets (Froidmont & Bartiaux-Thill 2004, Sujak et al. 2006). Although their main use is as livestock and poultry feed, they can also be used in the bakery industry for manufacturing a range of products (Erbas et al. 2005, Kohajdová et al. 2011). Addition of lupin flour (5%) in bread making can increase the tolerance index of dough (Dervas 1999). Human consumption of lupins is increasing (De Cortes-Sanchez et al. 2005).

Sujak et al. (2006) measured 29.5-35.6% crude protein, 5.5-8.6% oil, 11.6-14.1% crude fibre, 3.4-4.0% crude ash, 38.0-46.7% nitrogen free extract, 0.25-1.0% alkaloid among eight narrow-leafed lupin cultivars and 35.2-37.6% crude protein, 10.4-12.6% oil, 13.7-15.0% crude fibre, 3.7-4.1% crude ash, 33.5-34.3% nitrogen free extract, 0.37-0.40% alkaloid from two white lupin cultivars. They found that both species were suitable for animal and human nutrition and also for the production of protein supplementsin the food and feed industry, as they had high essential amino acid index (EAAI) and low alkaloid content.

Narrow-leafed lupin may need 104-114 days to mature in Serbia, with grain yield ranging from 1425-3005 kg ha^{-1} (Mihailovic et al. 2008). Campos-Andrada et al. (2008) found that mean seed yield of white lupin (cv. Estoril) was 2469 kg ha^{-1} and that of narrow-leafed lupin (cv. Tanjil) was 1350 kg ha^{-1} in Portugal. In a field trial in Finland, four narrow-leafed lupin cultivars (cv. Haags Blaue, cv. Boruta, cv. Boregine, cv. Sanabor) matured within 100-114 days and their grain yield ranged between 3280 and 4820 kg ha^{-1} (Stoddard et al. 2010).

As a legume crop, lupin growth and development depends on its biological nitrogen fixation (BNF) ability. BNF rate also depends on the presence and availability of soil mineral nitrogen status (Merbach et al. 2008). In narrow-leafed lupin, fixation of atmospheric nitrogen (% Ndfa) can decrease from 92% to 86% with increased application of 30 to 60 kg N ha^{-1} (Palmason et al. 1992).

Environmental factors (temperature, drought, soil pH and nutrient status) have a great impact on effective root-nodule association and BNF. Lower root zone temperature (7°C-12°C) severely inhibited nodulation in narrow-leafed lupin cultivars compared with optimum temperature (25°C) and at optimum temperature, the nodulation zone of the lupin root showed a high frequency of free root cap border cells and a distinct matrix of extracellular material that was significantly reduced or absent at low temperature (Peltzer et al. 2002). According to Bordeleau & Prevost (1994), the optimum temperature range for nodulation and nitrogen fixation was 20-30°C. High temperature may delay nodule initiation and development and ultimately reduces BNF.

Water stress also reduced yield and BNF (French & Turner 1991). Plant and rhizobial growth can be reduced under drought condition, causing nodulation failure and low BNF rates (Hungria & Vargas 2000). Seed yield of lupin genotypes may also be reduced from 24 to 66% under terminal drought condition (Palta et al. 2004).

Lupins can grow in a wide range of soils but their performances on distinct soils vary among species (Gladstones 1970). Generally, cultivated lupins prefer to grow on deep acidic to neutral sands or sandy loam soils, but white lupin has a stronger preference for loamy soil (White & French 2008, DeVarennes & Torres 2011).

The primary symbiont of cultivated lupins is *Bradyrhizobium* sp. (lupin). High temperature and moisture deficiency limits bradyrhizobial growth and survival in soil and is considered as a major cause of nodulation failure (Hungria & Vargas 2000).

Survival of *B.* sp. (lupin) depends on salinity, pH, $CaCO_3$ and antibiotics (Reza et al. 2001b). BNF depends on presence and availability of active *Bradyrhizobium* strains in soil and their suitability with host species (Graham & Vance 2000). BNF of lupins may range between 19-327 kg N ha^{-1} (Lupwayi & Kennedy 2007). Palmason et al. (1992) observed 195 kg ha^{-1} nitrogen fixation in narrow-leafed lupin in Korpa, Iceland.

Lupin growth and yield largely depends on an effective association with *Bradyrhizobium* and its associated symbiotic nitrogen fixation. Inoculation increased dry matter and grain yield in both narrow-leafed lupin (Merbach et al. 2008) and white lupin (Annicchiarico & Alami 2012). Nodulation and nitrogen fixation of lupin may vary with different bradyrhizobial inoculants resulting in a marked variation in nodule formation and dry matter yield (Abd-Alla 1999). *Bradyrhizobium* strains that are almost equally effective for lupins may not always show significant yield differences but some differences are obvious (Ayisi et al. 1992, Steinberga et al. 2008).

Although white lupin and narrow-leafed lupin have special interest as food and feed crops, their cultivation is limited on account of their low grain yields (Annicchiarico & Carroni 2009, Kohajdová et al. 2011). Their preference for specific soils and the difficulty of getting effective bradyrhizobial strains from soil are considered as the major causes of low yields (Annicchiarico & Alami 2012). Hence, it is necessary to screen the lupin cultivars according to their edaphic preference and inoculate them with appropriate bradyrhizobial strains.

1.1 Effect of soil

Soil may vary in their texture, pH and nutrient status and can play a significant role in lupin growth and development (White & Robson 1989, Esteban et al. 2003). Fine-textured alkaline soil reduced root penetration and nodule formation and ultimately dry matter yield (White & Robson 1989, Jessop et al. 1990). Plants grown in moderate limed soil produced lower grain and above-ground biomass yield than low limed condition (Annicchiarico & Alami 2012). This poor growth may be attributed to the high concentrations of bicarbonate, high clay content and iron deficiency of those fine-textured alkaline soils (Tang et al. 1995). Jessop et al. (1990) also reported that shoot, root and nodule dry mattter of lupin decreased at high lime content.

Lupin species differ greatly in response to soil pH. Shoot growth of both *L. angustifolius* and *L. albus* decreased at pH above 5-6, nodule mass of *L. angustifolius* decreased at pH above 7 and that of *L. albus* decreased at pH above 6 (Tang & Thomson 1996). The sharp decrease of nodulation in higher soil pH may also be attributed to poor adaptation of *Bradyrhizobium* to alkaline soil (Tang & Robertson 1995, Reza et al. 2001b). Soil pH > 6.5 may be unsuitable for narrow-leafed lupin growth and seed yield (French 2002). Tang & Robson (1993) also observed lower shoot and root fresh weight and reduced nodule formation in narrow-leafed lupin at pH > 6.

Plant height progressively reduced in lupins grown in a sequence of increasing lime concentrations (Jessop et al. 1990). Tang et al. (1993a) found that plant height and root length of *Lupinus* accessions were higher in acid soil and lower in alkaline soil. Root length of narrow-leafed and white lupins decreased at pH above 5 (Tang & Thomson 1996).

Alkaline soil or soil with high pH induced physical damage to the lupin root surface, disturbed root surface permeability and reduced root hair formation, resulting in impaired function of epidermal cells, decrease of root hair number, and reduced root surface area which impaired water and nutrient uptake from soil (Tang et al. 1993b, Tang et al. 1993c). Pathogenic microorganisms can easily attack damaged root surfaces and this may affect bradyrhizobial infection and nodule formation (Tang et al. 1993c).

Lupins are nitrogen-fixing plants that can grow in soils with poorly available nutrients (Esteban et al. 2003). White lupin is highly efficient in P uptake and in utilizing available sources of soil phosphorus. Many species can form cluster or proteoid roots that utilize sparingly available P sources in the rhizosphere (Neumann et al. 2000). Kerley (2000) found that cluster root formation of white lupin was only 17% of its total root biomass in neutral soil, but was over 30% in limed soil.

Lupins grown on alkaline clay or alkaline sand may show iron deficiency and slower shoot growth than those grown in acid loam or acid sand (Tang et al. 1995). The plants are very sensitive to Fe deficiency and show iron chlorosis, but the sensitivity varies among species, with *L. angustifolius*, *L. luteus* and *L. albus* being more sensitive than *L. pilosus*, *L. atlanticus* and *L. cosentinii* (Tang et al. 1995). Iron deficiency reduces leaf chlorophyll concentration but its main negative effect is to reduce nodulation (Tang et al. 2006). In Western Australia, iron deficiency in early growth depressed nodule initiation, resulting in decreased nodule number and dry weight, and decreased symbiotic nitrogen fixation by depressing leghaemoglobin concentration in nodules (Tang et al. 1990b). Addition of Fe to deficient soil increased nodulation and nitrogen fixation, resulting in higher shoot and root dry matter accumulations and increased nitrogen content (Abd-Alla 1999).

1.2 Effect of Bradyrhizobium

Rhizobiaceae are widely spread around the world, both naturally and in cultivation. Their presence in field soil plays a key role in legume productivity (Amarger 2001). In spite of this, effective soil rhizobia for a specific legume crop are often rare or absent. On the other hand, Rhizobiaceae-legume associations are very specific and some legumes may only form nodules with individual species, while others may form nodules with a range of species. This is attributable partly to the host-bacterium recognition through the exchange of their signal compounds, which induces differential gene expression in both partners (Broughton et al. 2000). However, inoculation with effective strains is highly desirable, as soil may contain many different Rhizobiaceae, most of which are symbiotically inactive with a particular legume (Deaker et al. 2004).

Legume inoculations have been used for over a century to introduce rhizobia or bradyrhizobia into the soil in order to increase crop productivity (Stephens & Rask 2000, Deaker et al. 2004). The aim is to provide sufficient numbers of viable effective bacteria that will induce rapid colonisation and early nodulation. The effectiveness of an individual Rhizobiaceae species on a specific legume primarily depends on symbiotic efficiency, but other factors like the ability to survive in adverse soil conditions or compatibility with other soil rhizobia in nodule production are also important (Date 2000, Graham & Vance 2000, Stephens & Rask 2000).

Inoculant quality depends on the number of viable bacteria available for infection of legume roots (Deaker et al. 2004). The use of the right carrier material may increase the survival capacity of bradyrhizobia in both storage and field condition. Strains of *B.* sp. (lupin), when transferred from broth culture to peat, acquired better adaptive capacity for long-term survival under nutrient-limited conditions by thickening their cell walls, which also makes the bacteria more resistant to other types of stress (Feng et al. 2002).

Inoculants may be applied directly to seed by dusting, slurry, pelleting and vacuum impregnation, or to the seed bed in liquid or granular form (Deaker et al. 2004). Seed inoculation is commonly done by the farmers immediately before sowing. Soil application of liquid inoculants also has proven an effective method of inoculation for many legumes (Danso et al. 1990, Brockwell et al. 1998). Seed inoculation allows substantial losses of viability of inoculant between inoculation and sowing, but it ensures higher rhizobial concentration in the vicinity of the seed, which is expected to lead to rapid colonisation and early nodulation. On the other hand, liquid inoculation ensures uniform distribution of rhizobia in the field, but their colonization in the rhizosphere may be relatively lower than the seed inoculation method (Brockwell et al. 1998). Regardless of inoculation method, the goal of inoculation for large-seeded legumes is to supply 10^5 to 10^6 bacteria seed^{-1} (Lupwayi et al. 2000).

Inoculation with effective bacteria in the Rhizobiaceae has a positive effect on legume yield. Although inoculation in general increased shoot, root and nodule dry weight of many legumes (including soybean, common bean and cowpea), the size of any yield increase mainly depended on the individual strain (Okereke et al. 2001, Figueiredo et al. 2008, Tahir et al. 2009, Lima et al. 2011).

Inoculation of lupin cultivars also resulted in significant increases in dry matter production and seed yield (Raza et al. 2000, Steinberga et al 2008). Abd-Alla (1999) conducted an experiment with six *Bradyrhizobium* strains (WPBS 3201D, WPBS 3211D, USDA 3040, USDA 3041, USDA 3042 and CB 2272) and three lupin species (*L. termis, L. albus* and *L. triticale*) and observed a significant increase in lupin shoot and root dry weight and nodule fresh weight with inoculation treatments compared to uninoculated treatments. He also observed that lupin species responded differently in terms of dry matter production when they were grown with different *Bradyrhizobium* strains. Instead of a single *Bradyrhizobium* strain, sometimes combined use of more than one inoculant may provide better seed and dry matter production in some lupin cultivars in field conditions (Raza et al. 2000).

Plant height of lupin may show a positive response to inoculation (Steinberga et al 2008). Increased shoot and root length in soybean with different *Bradyrhizobium* strains was also been previously reported (Tahir et al. 2009, Delić et al. 2010).

Pod number increased following rhizobial inoculations of legumes (Tahir et al. 2009) and this increase may also be an indicator of better seed yield (Ibrahim et al. 2011). Furthermore, seed pod^{-1} may respond to inoculation. Ali et al. (2000) observed significant increases in pod plant^{-1} and seed pod^{-1} following inoculation of three mungbean cultivars.

Nodulation of lupin depends on the presence of effective *Bradyrhizobium* strains. Lupin plants may fail to produce nodules if the soil is free from the effective organisms (Abd-Alla 1999, Delić et al. 2010). Even when the soil contains some effective bacteria, inoculation with *Bradyrhizobium* may increase nodulation in lupin (Raza et al. 2000).

Chlorophyll content of common bean was increased by rhizobial inoculation (Tajini et al. 2008). Inoculation increased nodulation and thus provided more nitrogen to the plants, resulting in higher leaf chlorophyll content. Nodulated soybean also showed higher chlorophyll content by SPAD spectrometer compared with non-nodulated plants (Vollmann 2011).

Bradyrhizobium inoculation increased nitrogen accumulation in lupin by increasing plant dry matter and nitrogen content. Although inoculation of lupin with different strains led to significant increases in total nitrogen accumulation in shoots and roots, the rate of accumulation varied among strains (Abd-Alla 1999). In an experiment with 15 lupin cultivars and four *B.* sp. (lupin), Reza et al. (2001) observed that N accumulation of those cultivars at 120 days after planting was between 1.06 to 3.49 mg plant^{-1} in uninoculated treatments and 1.75 to 7.00 mg plant^{-1} with different inoculation treatments. Similar increases in nitrogen accumulation following rhizobial inoculations have been observed in many other legumes including soybean and cowpea (Delić et al. 2010, Lima et al. 2011).

Biological nitrogen fixation depends on the proportion of symbiotically fixed nitrogen and total amount of nitrogen accumulated by plants during growth (Peoples et al. 1995). The efficiency of nitrogen accumulation by symbiotic association depends on the effectiveness of the bacterial strain. Biological nitrogen fixation rate is species-dependent and varies from legume to legume (Yang et al. 2010). White lupin can fix high amount of biological nitrogen (91% Ndfa) by effective bradyrhizobial inoculations (Carranca et al. 2009).

As lupin cultivation depends on soil characteristics, it is necessary to select the cultivars according to their soil preferences. Furthermore, crop yield depends on an effective *Bradyrhizobium*-lupin symbiosis, so it is necessary to identify a suitable strain for inoculation where lupin was not cultivated before.

1.3 Objectives

This study was undertaken to determine the effectiveness of different *Bradyrhizobium* strains with lupins under field conditions and also under greenhouse conditions using different potting media. The work was based on the following objectives:

1. to identify responses in growth and yield parameters of different narrow-leafed lupin cultivars with various *B.* sp. (lupin) strains under field conditions.
2. to identify responses in growth and yield parameters of different narrow-leafed and white lupin cultivars with various *B.* sp. (lupin) strains under greenhouse condition.
3. to quantify this response in relation to potting medium.
4. to determine N_2 fixation and competitive ability of selected strains.

2 MATERIALS AND METHODS

2.1.1 Plant materials

Three cultivars of narrow-leafed lupin, namely Haags Blaue, Boruta and Sonet were used in the field experiment. Haags Blaue and Boruta were supplied by the breeder, Saatzucht Steinach, Germany. The Polish cultivar Sonet was brought from Purolan Kartano, Tuusula, Finland. For the greenhouse experiment, white lupin cv. Ludic was bought from Purolan Kartano.

2.2 Inoculants

The bacterial strains used in this study were provided by HAMBI Culture Collection (HAMBI 2012). Freeze-dried strains were multiplied in Yeast Mannitol Broth (YMB) medium at 28°C prior to inoculation. The commercial lupin inoculant in peat, prepared from HAMBI 3118, was purchased from Elomestari OY (Kukkola, Finland).

Table 1. *B.* sp. (lupin) strains used in the experiments and their source.

Inoculant type	HAMBI code	Inoculation technique	Donor code	Donor information	Obtained from
Freeze-dried	3115	Liquid suspension	96b9	Nitragin company	HAMBI
Freeze-dried	3116	Liquid suspension	96b15	Nitragin company	HAMBI
Freeze-dried	3118	Liquid suspension	-	-	HAMBI
Commercial	3118	Slurry	-	Elomestari OY	Elomestari

2.3 Field experiment

The experiment was conducted at the Viikki experimental farm (60°13′N, 25°02′E) of the University of Helsinki, Finland during the period from 26 May 2010 to 30 August 2010. Soil of the experimental field was sandy loam with no previous history of lupin cultivation.

2.3.1 Weather conditions

The daily rainfall (mm) was quite low and daily average air temperature (°C) was moderately high during the growing season (Figure 1). Total rainfall accumulations (mm) of May, June, July and August were 69.8, 28.3, 33.0 and 113.6 mm respectively. Monthly average air temperature was comparatively low in May (11.3°C) and June (14.3°C) and higher in July (21.4°C) and August (17.4°C).

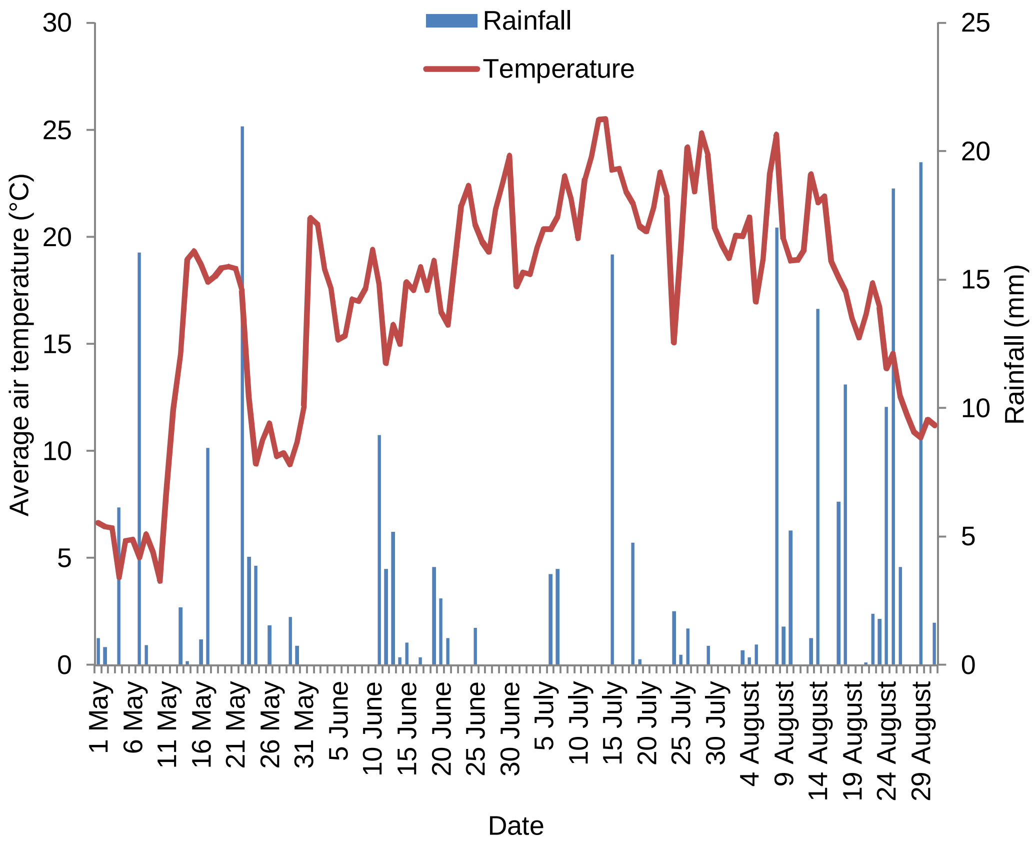


Figure 1. Daily average temperature (°C) and rainfall (mm) during the growing season in 2010, University of Helsinki, Viikki, Helsinki, Finland.

2.3.2 Experimental design

The experiment was laid out in a split-plot design with four replicates assigning five different *Bradyrhizobium* inoculant treatments, including an uninoculated control, to the main plots and three cultivars to the sub plots (Table 1). The sub plot size was 5m × 1.25 m = 6.25 m^2. The plot to plot distance was 0.25 m and that from replicate to replicate 2 m.

2.3.3 Seed sowing and inoculation

Prior to seed sowing, land was prepared by ploughing and harrowing, and the pre-emergence herbicide Stomp (Pendimethalin, 330 g a.i. L^{-1}) at the rate of 3 L ha^{-1} was applied for weed control. Each plot consisted of 10 rows 12.5 cm apart, where plant to plant distances were 5-6 cm resulting in a crop density of 125 plants m^{-2}. The commercial peat-based lupin inoculum treatment was applied to the seeds of that treatment before sowing. All seeds were sown on 26 May 2010 with a seed drill at 3-4 cm sowing depth. The three liquid inoculants (HAMBI 3115, HAMBI 3116 and

HAMBI 3118) were diluted with an appropriate amount of water and 3 L per sub plot were sprayed on to the seedlings and adjacent soil surface on 3 June 2010 after seed germination.

2.3.4 Crop management and harvesting

Phosphorus and potassium fertilizer at the rate of 12 kg P_2O_5 ha^{-1} and 20 kg K_2O ha^{-1} was applied with the seed drill at the time of sowing. The field was not irrigated during the entire growing season and depended solely on natural rainfall. Weed control was mainly done manually by pulling and uprooting. Focus ultra (Cycloxydin, 100 g a.i. L^{-1}) at the rate of 3 L ha^{-1} was applied on 3 June 2010 for controlling grass weeds. Haags Blaue and Sonet were harvested on 19 August 2010 and Boruta was harvested on 30 August 2010.

2.3.5 Measurements

Climate data were recorded at Vaisala WXT520 weather station located at Koetilantie 3, on the Viikki campus. Plant height was measured from 10 randomly selected plants at the pod-filling stage. Chlorophyll index was measured at flowering stage by using SPAD-502 chlorophyll meter. At maturity, a randomly selected 1.0 m^2 area was harvested from the 8 central rows of each sub plot. Root length, seed pod^{-1}, pod $plant^{-1}$ were measured immediately after harvesting. Shoot and root tissues were dried separately in an oven at 70°C for 72 hours before taking dry weight. Seeds were dried in an oven at 40°C for 72 hours.

2.4 Greenhouse Experiment

The experiment was conducted in the greenhouse of the University of Helsinki, Viikki campus, Helsinki (60°13′N, 25°02′E) from October 2010 to February 2011. Plants were grown in a greenhouse room with average day and night temperatures of 22°C and 18°C respectively. The experimental room was illuminated for 18 hours per day by using cool white fluorescent tubes.

2.4.1 Potting mixture (Soil type)

Three different types of potting medium, (1) soil, (2) 2 peat : 1 sand and (3) 1 peat : 2 sand were used in the experiment. Soil was collected from the Viikki experimental farm, which was characterised as sandy loam textured and having low pH. Plants were grown in tall and narrow 3 L plastic pots. Pots were labeled with individual codes and filled with specific potting mixture. To avoid soil compaction, vermiculite 10-15% by volume was added to the soil.

2.4.2 Experimental details

The experiment was conducted in a randomized complete block design with 4 replicates. Prior to seed sowing, pots were filled and arranged according to the randomization. All the seeds were sown on 30 October 2010. Five seeds were sown in each pot at 4-5 cm depth. The commercial inoculum treatment was applied to the seeds before sowing. Liquid inoculants were applied after seed germination on 5 November 2010. Plants were thinned on 9 November to 3 plants pot^{-1} for narrow-leafed lupin cultivars and 2 plants pot^{-1} for the white lupin cultivar.

Irrigation was done manually for the first two weeks and then automatic drip irrigation was set up. Initially, 70 ml water per pot was applied daily by the automatic irrigation, and later this was increased to 100 ml per pot. Plants were given nitrogen-free fertilizer containing 19.5% P, 23.6% K, 2.2% Mg, 0.03% B, 0.001% Co, 0.008% Cu, 0.17% Fe, 0.08% Mn, 0.005% Mo and 0.012% Zn, 0.15 g per pot. Biological pest controls were used throughout the whole experimental period. Mesurol 0.1% solution was applied on 12 and 20 January 2011 to control thrips.

Plants were harvested at the late pod filling stage. Haags Blaue and Sonet were harvested on 11 February, Boruta on 18 February and Ludic on 26 February.

2.4.3 Measurements

Chlorophyll index was measured by using SPAD-502 at the 90% flowering stage of each cultivar**.** Chlorophyll index of Haags Blaue and Sonet were measured on 27 December 2010, Boruta on 7 January 2011 and Ludic on 14 January 2011. After harvest, roots were washed carefully to ensure minimum nodule losses. Roots were separated from shoot portion and their lengths were measured. Nodules were counted and separated from the roots. All plant parts were dried separately in an oven at 70°C for 72 hours before dry weight was determined.

2.4.4 Nitrogen content and fixation

Shoot and root nitrogen concentration (% N) were measured from the plants grown in 2 peat : 1 sand potting medium. Oven-dried plant samples were milled with a fine grinder and their nitrogen concentration (% N) were analysed by the Dumas combustion method using a Vario Max CN analyser (Elementar, Germany). Shoot and root nitrogen contents (mg N) were estimated by the product of N concentration (% N) and dry matter of that plant part and finally total nitrogen content per plant (mg N plant^{-1}) was determined by adding nitrogen accumulation of those two parts (Rochester & Peoples 2005). Biological nitrogen fixation (BNF) was measured by the nitrogen difference method using the following formula (Unkovich et al. 2008):

$$\text{N fixed} = \text{N yield } N_2\text{-fixing plant} - \text{N yield reference plant}$$

Finally, the proportion of nitrogen derived from atmospheric N_2 (% Ndfa) was calculated by using the following formula (Peoples et al. 2009):

$$\%\ \text{Ndfa} = \left(\frac{\text{Nitrogen fixed}}{\text{Legume nitrogen}}\right) \times 100$$

Non-nodulated plants of the same cultivars as used in uninoculated treatments and grown in same potting medium were used as reference plants. This is considered ideal, because their physiology, morphology and anatomy are otherwise identical to those of the nodulated plants (Danso et al. 1992, Hardarson & Danso 1993).

2.5 Statistical analysis

All data collected were analysed using PASW (version 18.0, SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was done to test the significance of the effects of cultivar, inoculum, potting medium and their interactions, and the differences between means were tested by Tukey’s test.

3 RESULTS

3.1 Field experiment

Cultivars differed significantlyin shoot dry weight, root dry weight, seed dry weight, total plant dry weight, harvest index, plant height, root length and seed pod^{-1} (Appendix 1 and 2). Boruta had high values of shoot dry weight, root dry weight, seed dry weight, total plant dry weight, plant height, root length and seed pod^{-1}, while Haags Blaue had intermediate and Sonet had the lowest (Table 2). Highest harvest index was obtained from Haags Blaue and lowest from Boruta.

The different *Bradyrhizobium* treatments had significant effects on shoot dry weight, root dry weight, seed dry weight, total plant dry weight, harvest index, plant height, pod plant^{-1} and chlorophyll index (Appendix 1 and 2). Highest shoot dry weight, root dry weight, seed dry weight, total plant dry weight, pod plant^{-1} and chlorophyll index values were obtained from commercial inoculant and lowest from the uninoculated control treatment (Table 2).

Compared with the uninoculated control treatment, shoot, root, seed and total plant dry weight were increased by 47.9, 29.1, 68.6 and 55.2% by commercial inoculum, 26.8, 22.0, 25.1 and 25.8% by HAMBI 3115, 24.0, 18.3, 19.8 and 21.9% by HAMBI 3116 and 14.4, 11.9, 13.9 and 14.0% by HAMBI 3118. Similarly plant height, pod plant^{-1} and chlorophyll content were also significantly increased 10.7, 50.6 and 20.8% by commercial inoculum, 5.6, 22.5 and 14.3% by HAMBI 3115, 5.1, 16.8 and 9.8% by HAMBI 3116, 3.6, 10.7 and 5.7% by HAMBI 3118.

The field experiment did not show any significant interaction between *Bradyrhizobium* treatments and narrow-leafed lupin cultivar (Appendix 1 and 2).

Table 2. The effect of different *Bradyrhizobium* inoculations on shoot dry weight, root dry weight, seed dry weight, total plant dry weight, harvest index, plant height, root length, seed pod^{-1}, pod plant^{-1} and chlorophyll index in 3 narrow-leafed lupin cultivars in a field experiment.

Treatment	Shoot dry weight (g m^{-2})	Root dry weight (g m^{-2})	Seed dry weight (g m^{-2})	Total dry weight (g m^{-2})	Harvest index (%)	Plant height (cm)	Root length (cm)	Seed pod^{-1}	Pod plant^{-1}	Chlorophyll index (SPAD unit)
Cultivar										
Haags blaue	169b	21.7b	147b	337b	43.4a	35.0b	18.6b	3.45b	4.50	28.9
Boruta	213a	25.4a	160a	398a	40.0b	37.8a	19.2a	4.75a	4.56	30.2
Sonet	156b	19.9b	130c	306c	42.4a	34.7b	18.1c	3.44b	4.27	27.3
SE	5.3	0.61	3.4	9	0.37	0.50	0.11	0.012	0.10	0.84
Bradyrhizobium										
Control	146c	19.2c	116c	281c	41.3b	34.1b	18.6	3.85	3.70c	26.1b
Commercial	216a	24.8a	196a	437a	44.9a	37.8a	19.0	3.92	5.57a	31.6a
HAMBI 3115	185b	23.5ab	145b	354b	41.2b	36.0ab	18.5	3.88	4.53b	29.9ab
HAMBI 3116	181b	22.7ab	139b	343b	40.7b	35.9ab	18.5	3.87	4.32b	28.7ab
HAMBI 3118	167bc	21.5bc	132bc	321bc	41.4b	35.3ab	18.6	3.88	4.10bc	27.6ab
SE	6.9	0.79	4.4	11	0.47	0.65	0.14	0.016	0.12	1.1

Numbers followed by the same letter within a column are not significantly different by Tukey’s test.

3.2 Greenhouse experiment

3.2.1 Effect of species on growth and yield components of lupin

Species showed significant differences in all the growth and yield parameters that were measured in the greenhouse experiment (Appendix 3 and 4). White lupin cultivar Ludic had significantly higher shoot, root, nodule and plant dry matter yield compared with any of the three narrow-leafed lupin cultivars (Table 3). Plant height, nodule number and chlorophyll index were also significantly higher in the white lupin (Table 3).

3.2.2 Effect of cultivar on growth and yield components of lupin

There were significant differences between the three narrow-leafed lupin cultivars in all the parameters of greenhouse experiment (Appendix 3 and 4). The highest shoot dry weight, root dry weight, nodule dry weight, total plant dry weight, plant height and chlorophyll index were found in Boruta and the lowest values in Sonet (Table 3). Root length and nodule numbers were higher in Haags Blaue and Boruta and lower in Sonet (Table 3).

3.2.3 Effect of *Bradyrhizobium* inoculant on growth and yield components of lupin

Bradyrhizobium inoculation had a significant effect on all the growth and yield components except root length (Appendix 3 and 4). Compared with the control, shoot and root dry matter increased by 119.8% and 45.8% with the commercial inoculant, 141.9% and 64.4% by HAMBI 3115, 120.0% and 49.0% by HAMBI 3116, 117.1% and 50.3% by HAMBI 3118. Total plant dry weight was increased between 114.9 and 136.2% with different bradyrhizobial inoculation.

Highest shoot, root and nodule dry matter were obtained from the HAMBI 3115 treatment whereas the lowest values were obtained in the non-inoculated control treatment (Table 3). Total plant dry matter significantly increased by 136.2%, 116.0%, 114.9% and 112.5% with HAMBI 3115, commercial inoculant, HAMBI 3116 and HAMBI 3118 respectively.

Plant height significantly increased by 46.0%, 41.0%, 38.6% and 38.3% with HAMBI 3115, HAMBI 3116, commercial inoculant and HAMBI 3118 respectively. More nodules were observed in *Bradyrhizobium* inoculation treatments (31.7-33.0 nodules plant^{-1}) compared with control (4.4 nodules plant^{-1}). Chlorophyll content significantly increased by 38.5%, 32.7% and 30.4% and 29.0% with HAMBI 3115, HAMBI 3116, HAMBI 3118 and commercial inoculant respectively.

3.2.4 Effect of potting medium on growth and yield components of lupin

Potting medium showed significant effect on all the yield and yield components (Appendix 3 and 4). Compared with soil, potting medium 2 peat : 1 sand and 1 peat : 2 sand showed reduced shoot, root, nodule and total plant dry weight by 43.1 and 47.3%, 9.1 and 14.2% , 33.2 and 38.9%, 40.9 and 45.2% respectively.

Plant height decreased by 11.27-11.43% but root length increased by 13.60-15.08% in the peat-sand potting media compared with soil. Different peat-sand media significantly decreased nodule number and chlorophyll index (Table 3). For instance, in 2 peat : 1 sand and 1 peat : 2 sand nodule number was reduced by 7.18 and 23.95% and chlorophyll content was decreased by 12.27 and 15.99% respectively.

In general, all the parameters except root length were significantly decreased in the two peat and sand media compared with soil (Table 3).

Table 3. The effect of *Bradyrhizobium* inoculations and potting media on shoot dry weight, root dry weight, nodule dry weight, total plant dry weight, plant height, root length, nodule number and chlorophyll index in 3 narrow-leafed lupin and 1 white lupin cultivar in a greenhouse experiment.

Treatment	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Nodule dry weight (g plant^{-1})	Total plant dry weight (g plant^{-1})	Plant height (cm)	Root length (cm)	Nodule number	Chlorophyll index (SPAD unit)
Cultivar								
Haags Blaue	2.21c	0.281b	0.065c	2.56c	49.0bc	37.3a	25.1b	37.0c
Sonet	1.79c	0.170c	0.043d	2.00c	47.3c	32.6b	26.0b	35.1c
Boruta	4.04b	0.307b	0.118b	4.47b	51.4b	35.2a	21.9c	39.8b
Ludic	12.79a	0.977a	0.417a	14.19a	84.8a	30.7b	33.7a	46.2a
SE	0.15	0.010	0.004	0.16	0.65	0.63	0.69	0.67
Bradyrhizobium								
Control	2.60c	0.306c	0.054b	2.96c	43.8c	34.7	4.4b	31.3c
Commercial	5.77ab	0.446b	0.182a	6.40ab	60.7b	33.5	32.4a	40.4b
HAMBI 3115	6.29a	0.503a	0.198a	6.99a	63.9a	33.9	31.8a	43.4a
HAMBI 3116	5.72ab	0.456b	0.185a	6.37ab	61.7ab	33.7	31.7a	41.6ab
HAMBI 3118	5.65b	0.460ab	0.184a	6.29b	60.5b	34.1	33.1a	40.8ab
SE	0.16	0.011	0.005	0.18	0.73	0.70	0.77	0.75
Potting media								
Soil	7.45a	0.471a	0.211a	8.14a	63.2a	31.0b	29.8a	43.6a
2 peat : 1 sand	4.24b	0.428b	0.141b	4.81b	56.1b	35.2a	27.7b	38.3b
1 peat : 2 sand	3.93b	0.404b	0.129b	4.46b	56.0b	35.7a	22.7c	36.7b
SE	0.13	0.009	0.004	0.14	0.57	0.54	0.59	0.58

Numbers followed by the same letter within a column are not significantly different by Tukey’s test.

3.2.5 Interaction of inoculum and potting medium

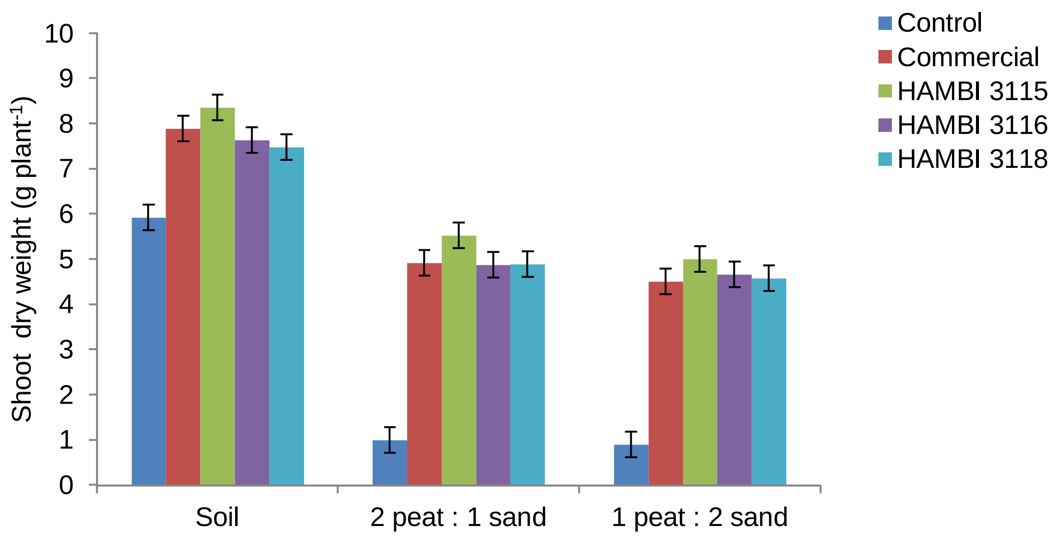
The results showed significant *Bradyrhizobium* × potting medium interaction effects on shoot dry weight, root dry weight , nodule dry weight, plant dry weight, plant height, nodule number and chlorophyll index (Appendix 3 and 4).

The difference between inoculated and uninoculated plants in peat-sand mixtures was always large, and that in soil was relatively small (Figure 2). In the soil treatment, many nodules formed, but none were found in the two peat-sand mixtures. In all cases except nodule number, values were highest in HAMBI 3115 than in the other 3 inoculation treatments, although the differences were not significant.

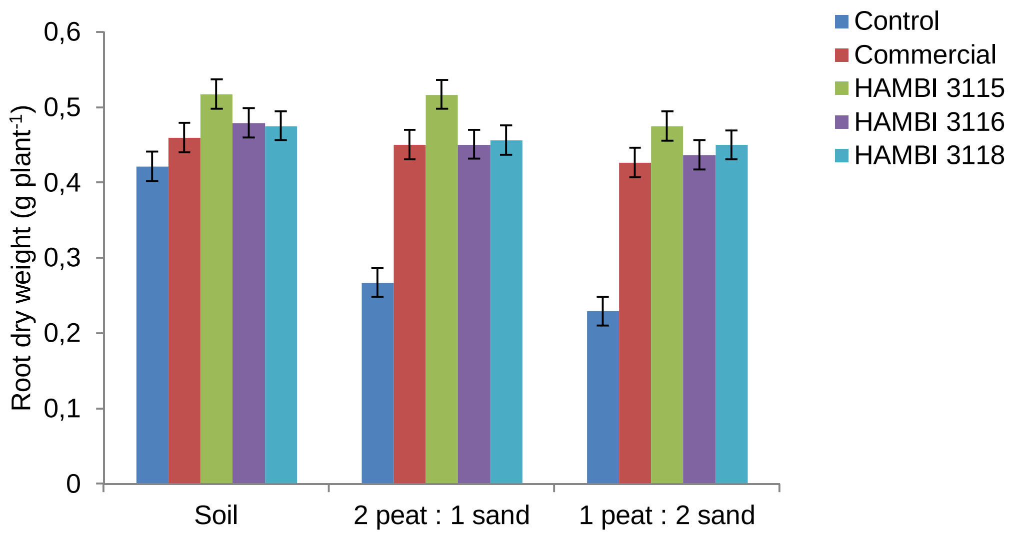
Greater shoot, root, nodule and plant dry weight were recorded in treatment involving in different *Bradyrhizobium* inoculation and soil (Figure 2a-d). Highest shoot, root, nodule and plant dry weight were obtained from the combination of *Bradyrhizobium* strain HAMBI 3115 and soil and the lowest were obtained from non-inoculated control treatment in 1 peat : 2 sand potting media (Figure 2a-d).

Plant height and chlorophyll index were also highest in the combination of HAMBI 3115 and soil and lowest in the combination of uninoculated control treatment and 1 peat : 2 sand potting media (Figure 2e and g). More nodules were observed in the combination of HAMBI 3118 and 2 peat : 1 sand potting medium and less in uninoculated control plants those grown in both peat-sand potting media (Figure 2f).

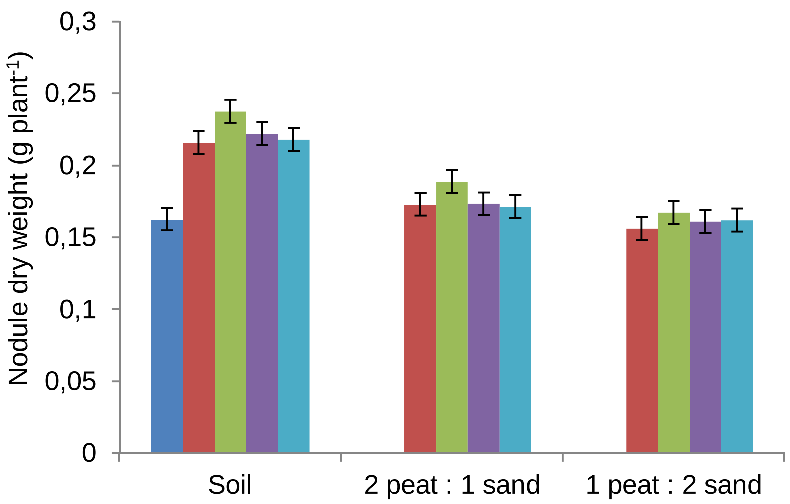
(a)



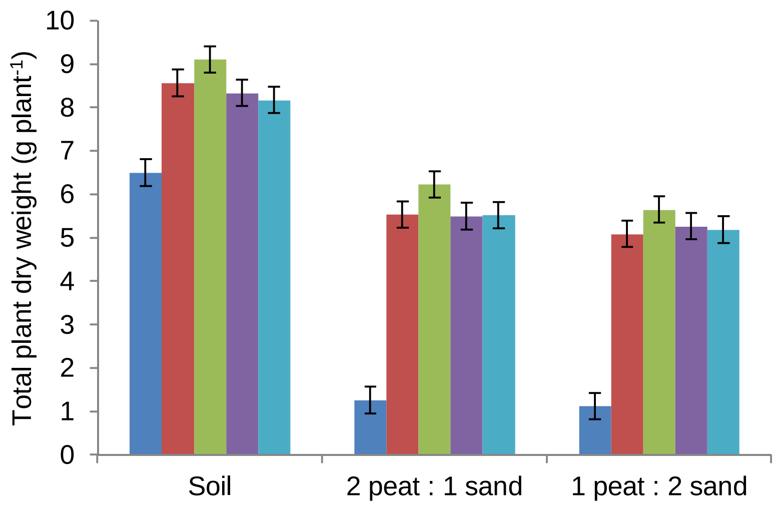
(b)


Root dry weight (g plant-1)
0,6
0,5
0,4
0,3
0,2
0,1
0
Soil
2 peat : 1 sand
1 peat : 2 sand
Control
Commercial
HAMBI 3115
HAMBI 3116
HAMBI 3118

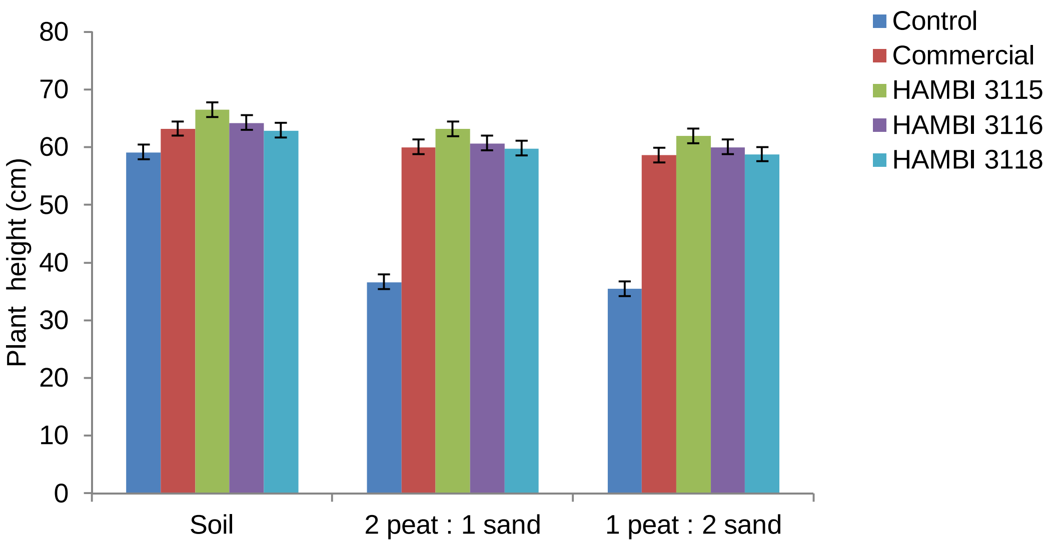
(c)


Nodule dry weight (g plant-1)
0,3
0,25
0,2
0,15
0,1
0,05
0
Soil
2 peat : 1 sand
1 peat : 2 sand

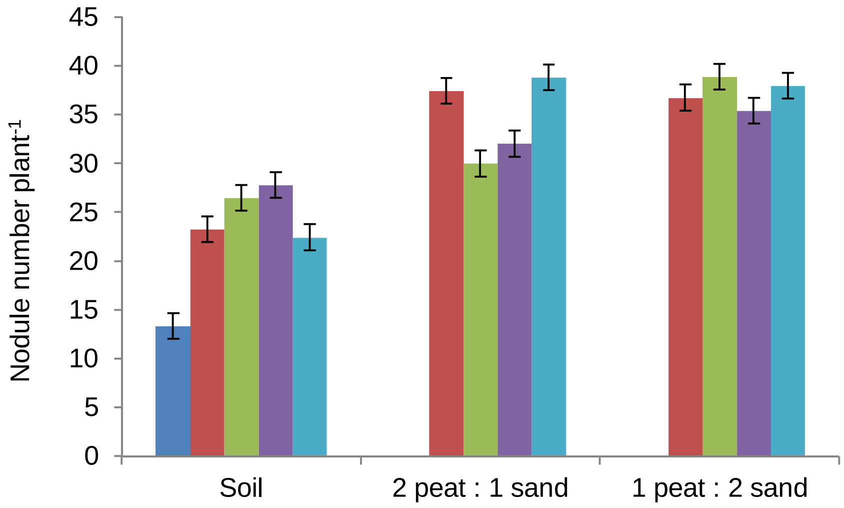
(d)


Total plant dry weight (g plant-1)
10
9
8
7
6
5
4
3
2
1
0
Soil
2 peat : 1 sand
1 peat : 2 sand

(e)


80
70
60
50
40
30
20
10
0
Plant height (cm)
Soil
2 peat : 1 sand
1 peat : 2 sand
Control
Commercial
HAMBI 3115
HAMBI 3116
HAMBI 3118

(f)


45
40
35
30
25
20
15
10
5
0
Nodule number plant-1
Soil
2 peat : 1 sand
1 peat : 2 sand

(g)

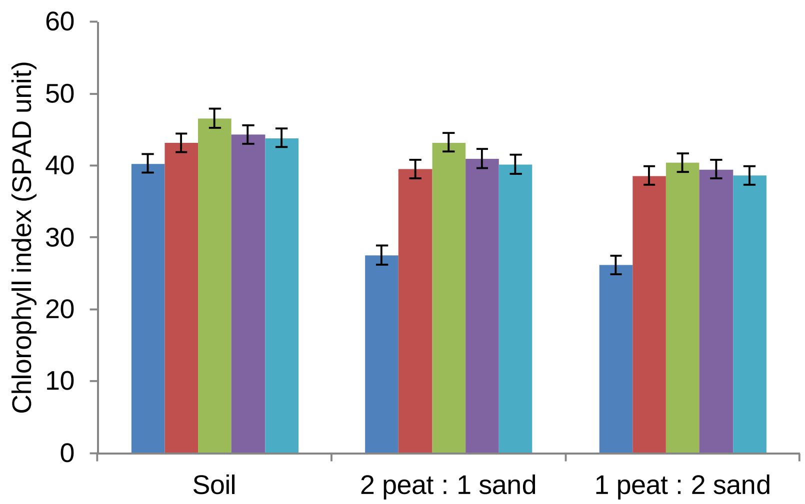

60
50
40
30
20
10
0
Chlorophyll index (SPAD unit)
Soil
2 peat : 1 sand
1 peat : 2 sand

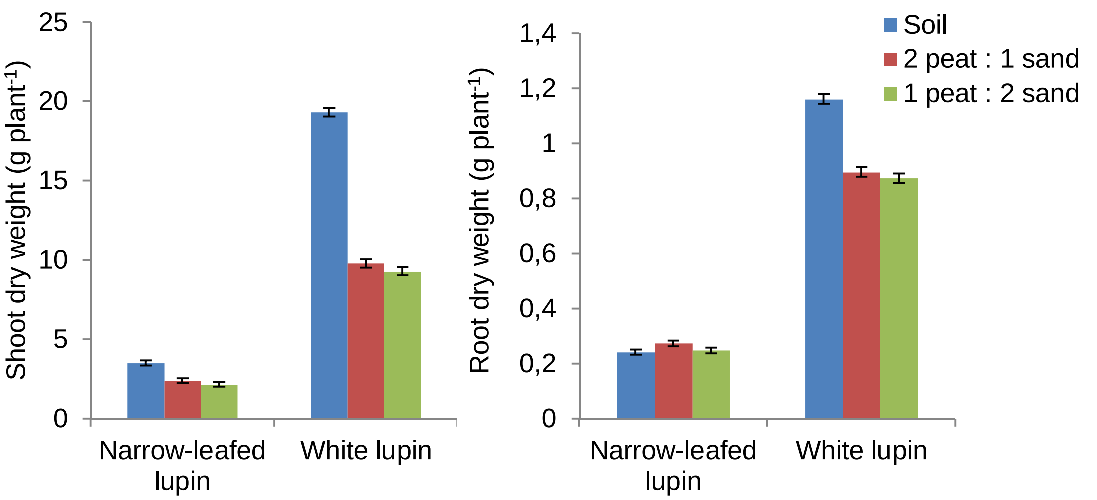
Figure 2. Effects of *Bradyrhizobium* inoculant and potting medium on: (a) shoot dry weight, (b) root dry weight, (c) nodule dry weight, (d) total plant dry weight, (e) plant height, (f) nodule number and (g) chlorophyll index in a greenhouse experiment. Error bars show ± 1 SE. Means of 4 replicates of 4 cultivars.

3.2.6 Interaction of species and potting medium

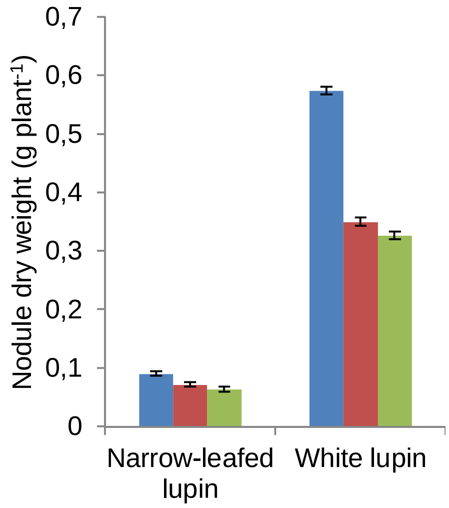
The lupin species × potting medium interaction was significant in all parameters measured in the greenhouse experiment (Appendix 3 and 4). Maximum shoot, root, nodule and plant dry weight, plant height and chlorophyll index were obtained from white lupin grown in soil and the minimum were obtained from narrow-leafed lupin and 1 peat : 2 sand (Figure 3a-e, h). Narrow-leafed lupin grown in 2 peat : 1 sand medium produced longest roots while the shortest was observed in white lupin grown in 1 peat : 2 sand potting medium (Figure 3f). Maximum numbers of nodules were produced by white lupin in 1 peat : 2 sand medium and minimum numbers were obtained from narrow-leafed lupin grown in soil (Figure 3g).

In both species, shoot, root, nodule and plant dry weight, plant height and chlorophyll index were highest in the soil medium, lower in 2 peat : 1 sand potting medium and lowest in 1 peat : 2 sand potting medium. The difference was always large in white lupin but less in narrow-leafed lupin. In narrow-leafed lupin, the values of these parameters were similar in all 3 potting media, but in white lupin they were much higher in soil than in the two peat-sand potting media. Roots of narrow-leafed lupin were longer in both peat-sand potting media than in soil . Nodule numbers were almost the same in both peat-sand potting media in narrow-leafed lupin but differed in white lupin.

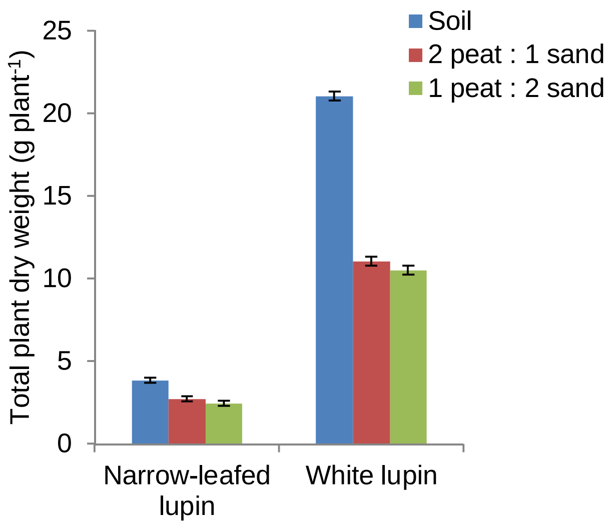
(a) (b)



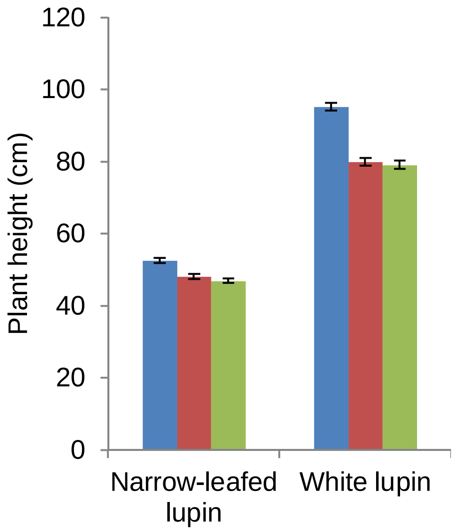
(c)


Nodule dry weight (g plant-1)
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
Narrow-leafed lupin
White lupin

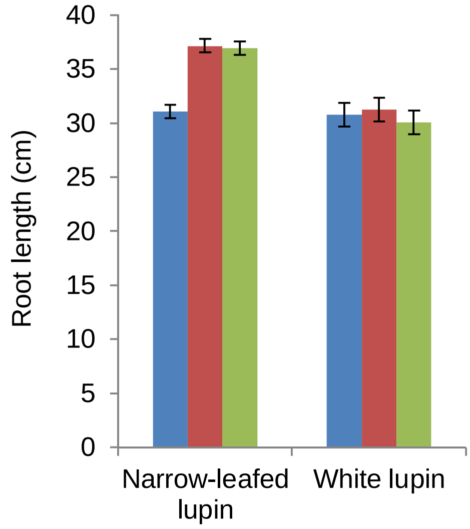
(d)


Total plant dry weight (g plant-1)
25
20
15
10
5
0
Narrow-leafed lupin
White lupin
Soil
2 peat : 1 sand
1 peat : 2 sand

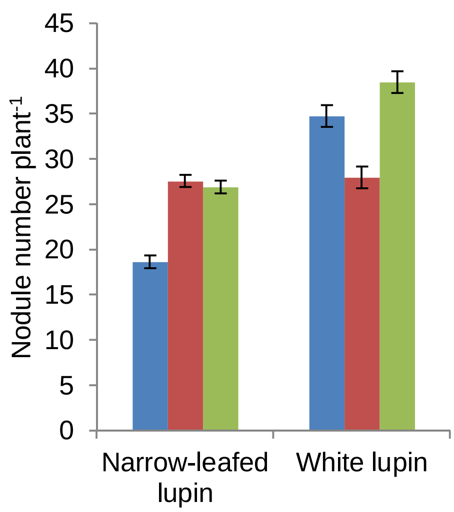
(e)


Plant height (cm)
120
100
80
60
40
20
0
Narrow-leafed lupin
White lupin

(f)


Root length (cm)
40
35
30
25
20
15
10
5
0
Narrow-leafed lupin
White lupin

(g)


Nodule number plant-1
45
40
35
30
25
20
15
10
5
0
Narrow-leafed lupin
White lupin

(h)

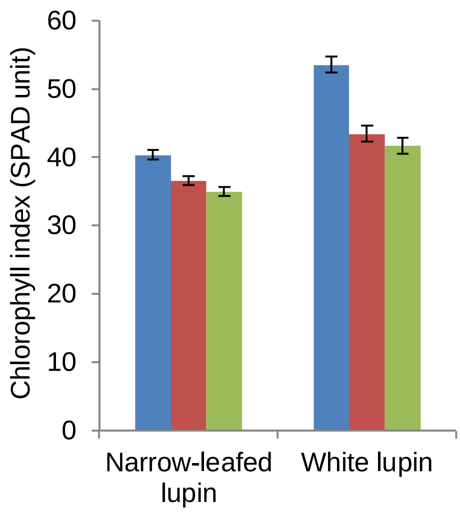

Chlorophyll index (SPAD unit)
60
50
40
30
20
10
0
Narrow-leafed lupin
White lupin

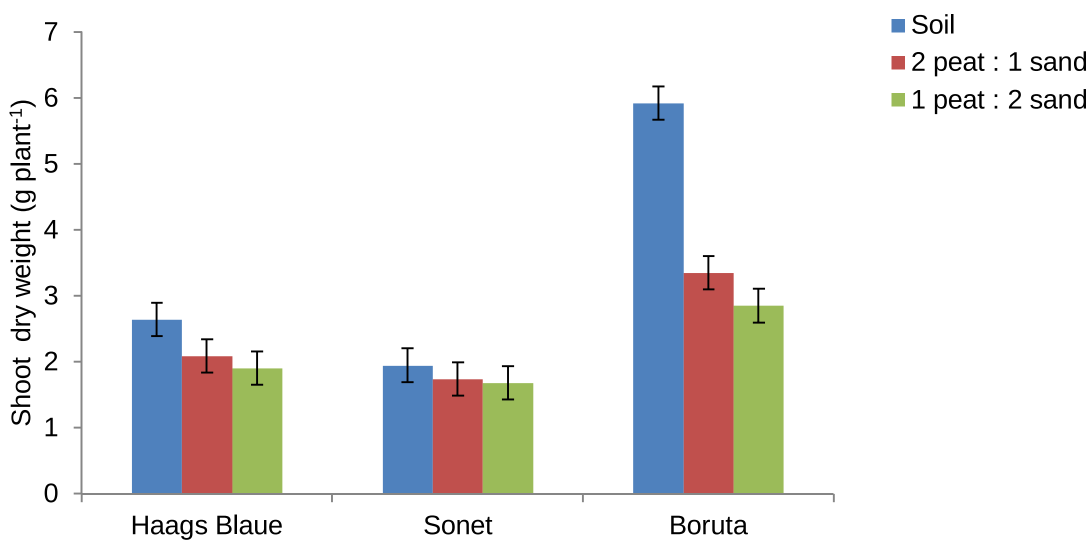
Figure 3. Effects of potting medium on: (a) shoot dry weight, (b) root dry weight, (c) nodule dry weight, (d) total plant dry weight, (e) plant height, (f) root length, (g) nodule number and (h) chlorophyll index in narrow-leafed lupin (3 cultivars) and white lupin (1 cultivar) in a greenhouse experiment. Error bars show ± 1 SE. Means of 4 replicates of 5 inoculation treatments.

3.2.7 Interaction of cultivar and potting medium

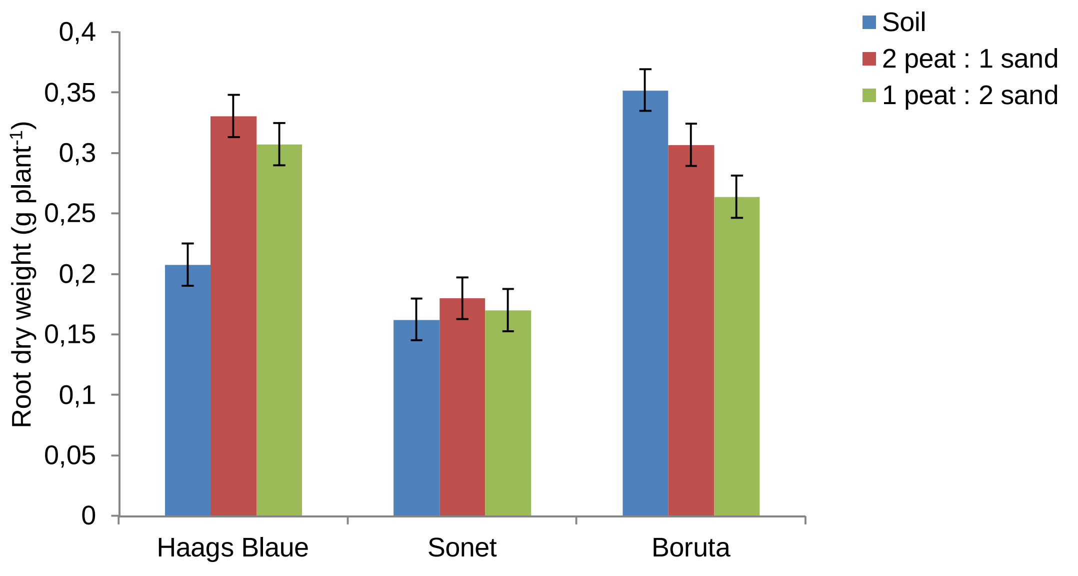
The interaction between potting medium and narrow-leafed lupin cultivar was significant for shoot dry weight, root dry weight, nodule dry weight, total plant dry weight and nodule number (Appendix 3 and 4). The highest shoot, root, nodule and plant dry weights were obtained from Boruta in soil and the lowest values were obtained from Sonet in 1 peat : 2 sand medium (Figure 4a-d). Maximum number of nodules was produced from Haags Blauein 2 peat : 1 sand medium and minimum number of nodules was produced from Sonet in soil (Figure 4e).

Narrow-leafed lupin cultivars showed higher shoot, nodule and plant dry weight in soil, while lower in 2 peat : 1 sand and lowest in 1 peat : 2 sand potting medium, but these differences were large only in Boruta and small in Haags Blaue and Sonet. Root dry weight of cultivars Haags Blaue and Sonet was lower in soil than in both peat-sand potting media, but that of Boruta was highest in soil and gradually decreased in 2 peat : 1 sand and 1 peat : 2 sand potting medium. Nodule number was always lowest in soil medium and higher in 2 peat : 1 sand or 1 peat : 2 sand potting medium in all 3 narrow-leafed lupin cultivars.

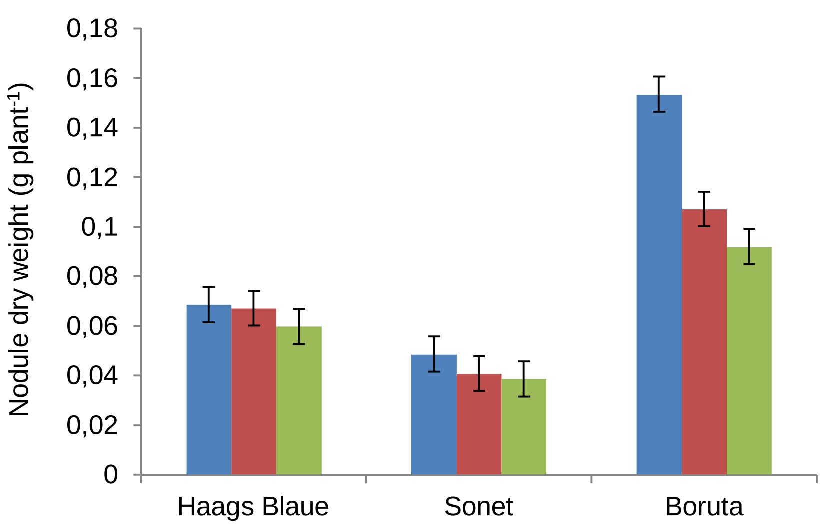
(a)



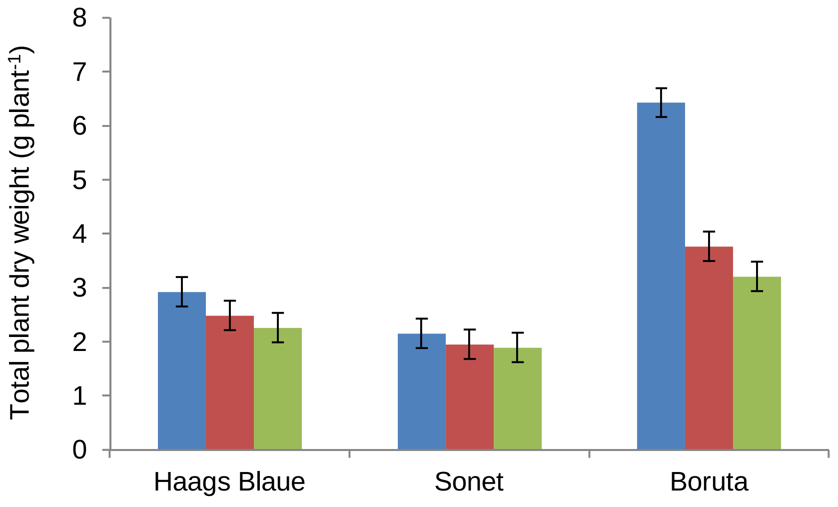
(b)


Soil
2 peat : 1 sand
1 peat : 2 sand
Root dry weight (g plant-1)
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
Haags Blaue
Sonet
Boruta

(c)


Nodule dry weight (g plant-1)
0,18
0,16
0,14
0,12
0,1
0,08
0,06
0,04
0,02
0
Haags Blaue
Sonet
Boruta

(d)


Total plant dry weight (g plant-1)
8
7
6
5
4
3
2
1
0
Haags Blaue
Sonet
Boruta

(e)

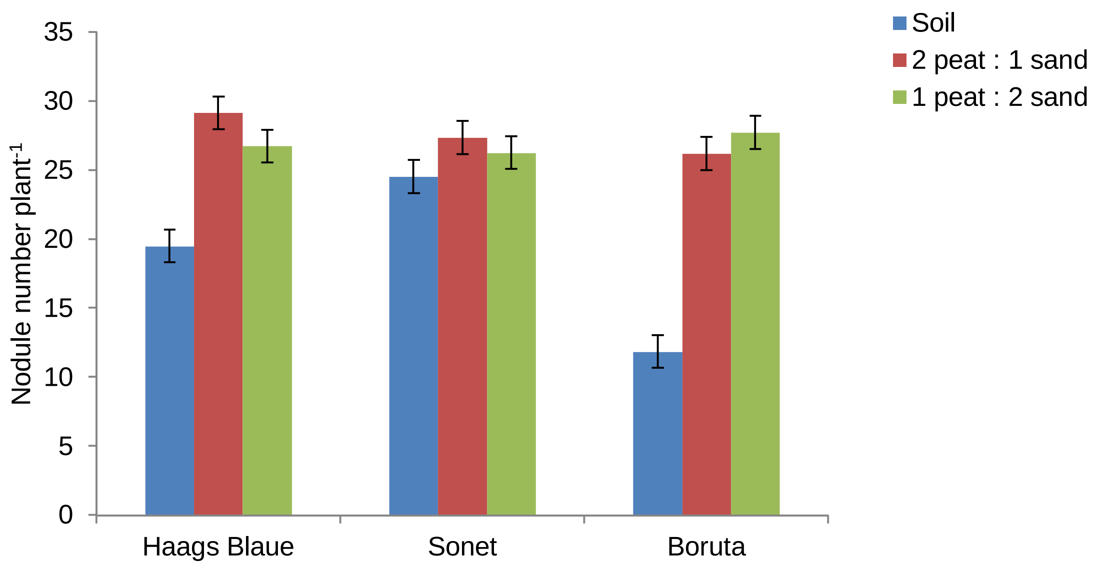
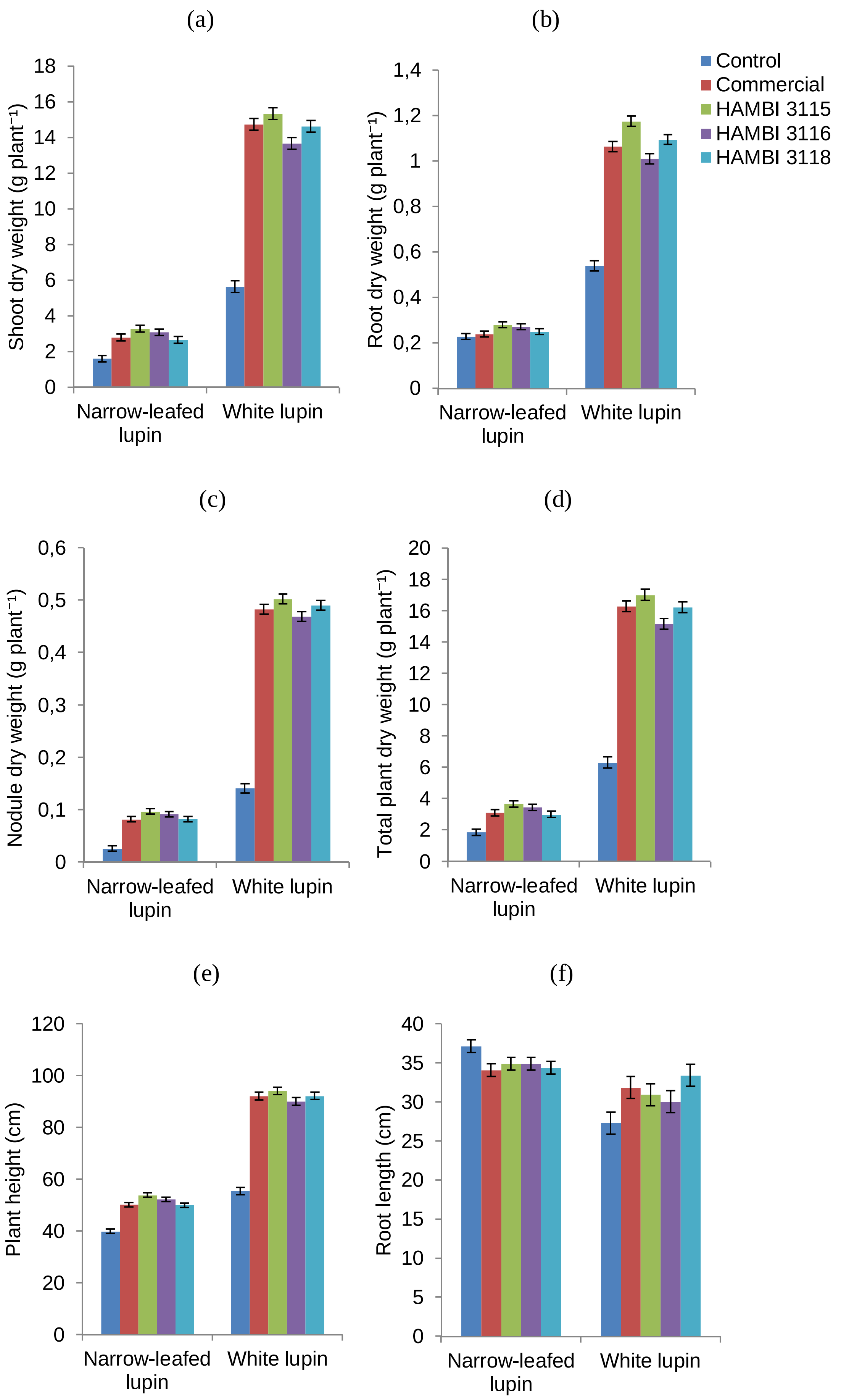


Figure 4. Effects of potting medium on: (a) shoot dry weight, (b) root dry weight, (c) nodule dry weight, (d) total plant dry weight and (e) nodule number in 3 narrow-leafed lupin cultivars in a greenhouse experiment. Error bars show ± 1 SE. Means of 4 replicates of 5 inoculation treatments.

3.2.8 Interaction of lupin species and inoculum

The lupin species × inoculum interaction was significant for all parameters measured in the greenhouse experiment (Appendix 3 and 4). Highest shoot dry weight, root dry weight, nodule dry weight, plant dry weight, plant height, nodule number and chlorophyll index were obtained from the combination of HAMBI 3115 strain and white lupin while the lowest were obtained from narrow-leafed lupins and uninoculated control treatment (Figure 5a-e, g and h).

The differences between inoculated and uninoculated plants in shoot, root, nodule and plant dry weight plant height and chlorophyll index were always large in white lupin but relatvely low in narrow-leafed lupin. Uninoculated narrow-leafed lupin produced the longest roots while uninoculated white lupin produced the shortest (Figure 5f). Inoculation with HAMBI 3118 or its commercial peat inoculum produced more nodules than the other two inoculants in narrow-leafed lupin, but the opposite was observed in white lupin (Figure 5g).



(a)
(b)
Control
Commercial
HAMBI 3115
HAMBI 3116
HAMBI 3118
Shoot dry weight (g plant⁻¹)
0
2
4
6
8
10
12
14
16
18
Root dry weight (g plant⁻¹)
0
0,2
0,4
0,6
0,8
1
1,2
1,4
Narrow-leafed lupin
White lupin
(c)
(d)
Nodule dry weight (g plant⁻¹)
0
0,1
0,2
0,3
0,4
0,5
0,6
Total plant dry weight (g plant⁻¹)
0
2
4
6
8
10
12
14
16
18
20
Narrow-leafed lupin
White lupin
(e)
(f)
Plant height (cm)
0
20
40
60
80
100
120
Root length (cm)
0
5
10
15
20
25
30
35
40
Narrow-leafed lupin
White lupin

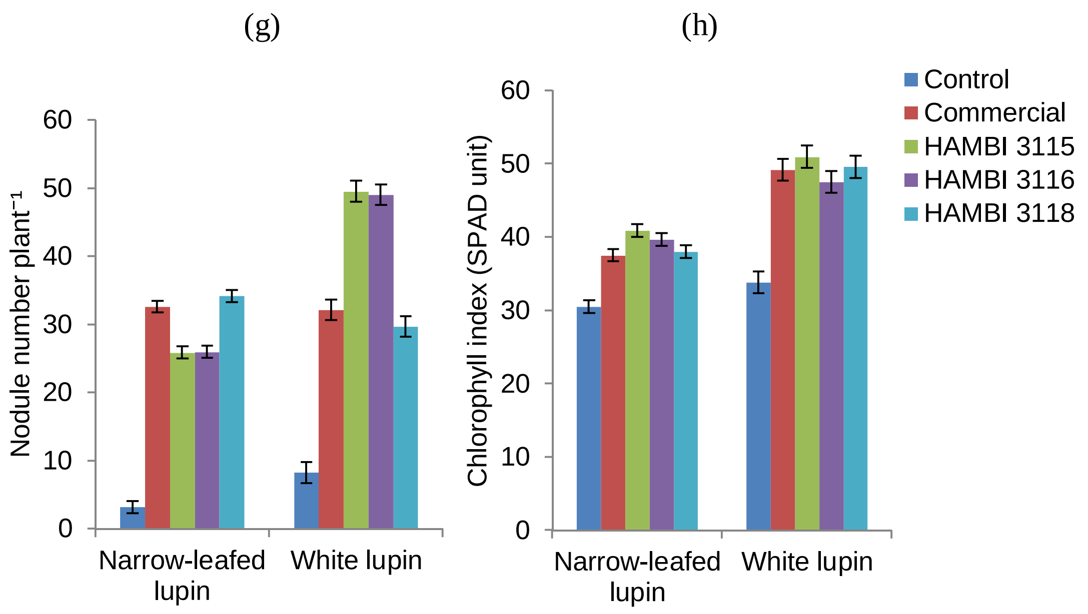


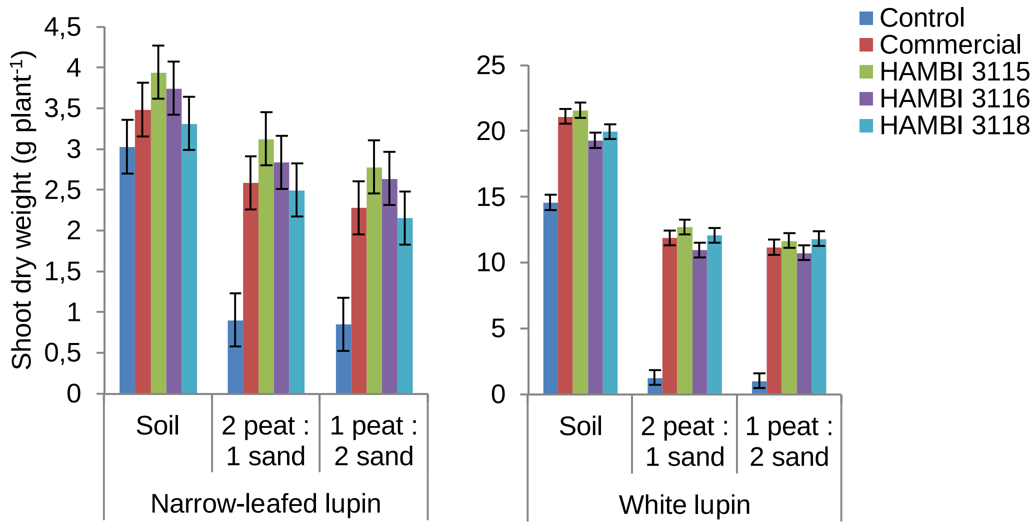
Figure 5. Effects of *Bradyrhizobium* inoculation on: (a) shoot dry weight, (b) root dry weight, (c) nodule dry weight, (d) total plant dry weight, (e) plant height, (f) root length (g) nodule number and (h) chlorophyll index in narrow-leafed lupin (3 cultivars) and white lupin (1 cultivar) in a greenhouse experiment. Error bars show ± 1 SE. Means of 4 replicates of 3 potting media.

3.2.9 Interaction of lupin species, potting medium and inoculum

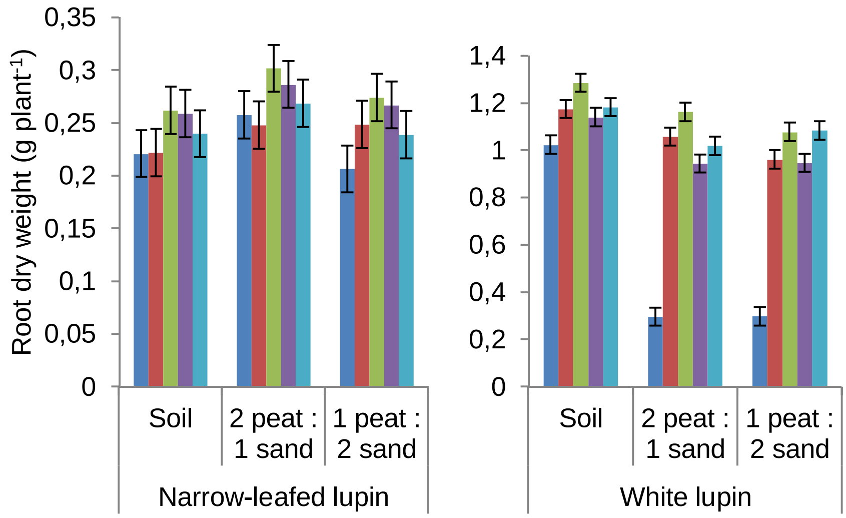
The three-way interaction between species, potting medium and *Bradyrhizobium* was significant in shoot dry weight, root dry weight, nodule dry weight, plant dry weight, plant height and nodule number (Appendix 3 and 4). Highest shoot dry weight, root dry weight, nodule dry weight, plant dry weight, plant height and nodule number were obtained from the combination of white lupin, soil and HAMBI 3115 strain and the lowest were obtained from uninoculated narrow-leafed lupin that grown in 1 peat : 2 sand potting medium. (Figure 6a-f).

In narrow-leafed lupin, HAMBI 3116 > HAMBI 3118 in most measures, but in white lupin, the opposite held true. The dry matter values of uninoculated white lupin in peat-sand potting media are much lower with respect to those of inoculated counterparts than those of white lupin in soil or blue lupin in any potting medium.

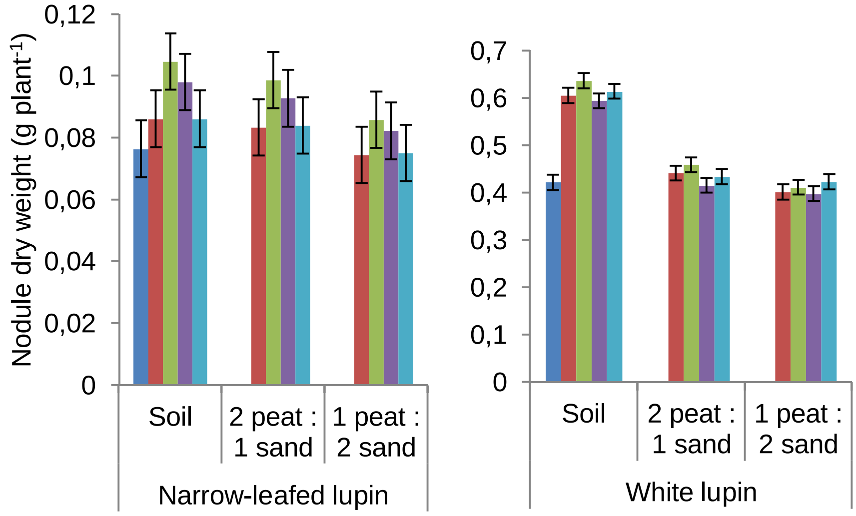
(a)


Shoot dry weight (g plant-1)
0
0,5
1
1,5
2
2,5
3
3,5
4
4,5
Soil
2 peat : 1 sand
1 peat : 2 sand
Narrow-leafed lupin
0
5
10
15
20
25
White lupin
Control
Commercial
HAMBI 3115
HAMBI 3116
HAMBI 3118

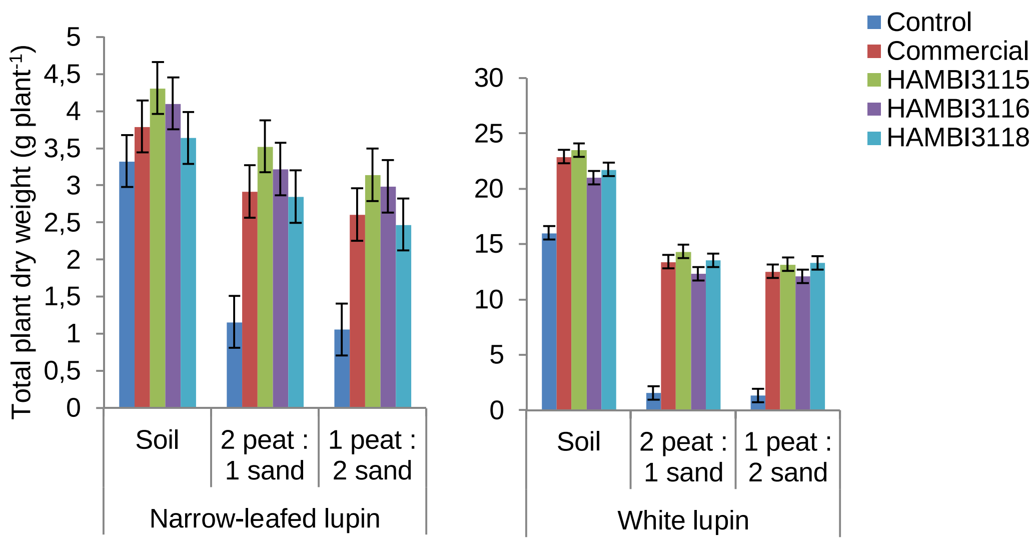
(b)


Root dry weight (g plant-1)
0
0,05
0,1
0,15
0,2
0,25
0,3
0,35
Soil
2 peat : 1 sand
1 peat : 2 sand
Narrow-leafed lupin
0
0,2
0,4
0,6
0,8
1
1,2
1,4
White lupin

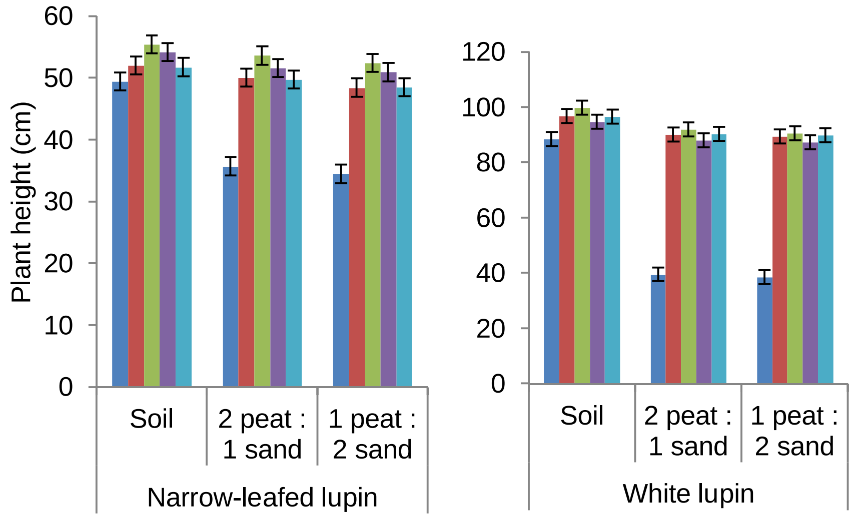
(c)


Nodule dry weight (g plant-1)
0
0,02
0,04
0,06
0,08
0,1
0,12
Soil
2 peat : 1 sand
1 peat : 2 sand
Narrow-leafed lupin
0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
White lupin

(d)


Total plant dry weight (g plant-1)
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
30
25
20
15
10
5
0
Soil
2 peat : 1 sand
1 peat : 2 sand
Narrow-leafed lupin
White lupin
Control
Commercial
HAMBI3115
HAMBI3116
HAMBI3118

(e)


Plant height (cm)
60
50
40
30
20
10
0
120
100
80
60
40
20
0
Soil
2 peat : 1 sand
1 peat : 2 sand
Narrow-leafed lupin
White lupin

(f)

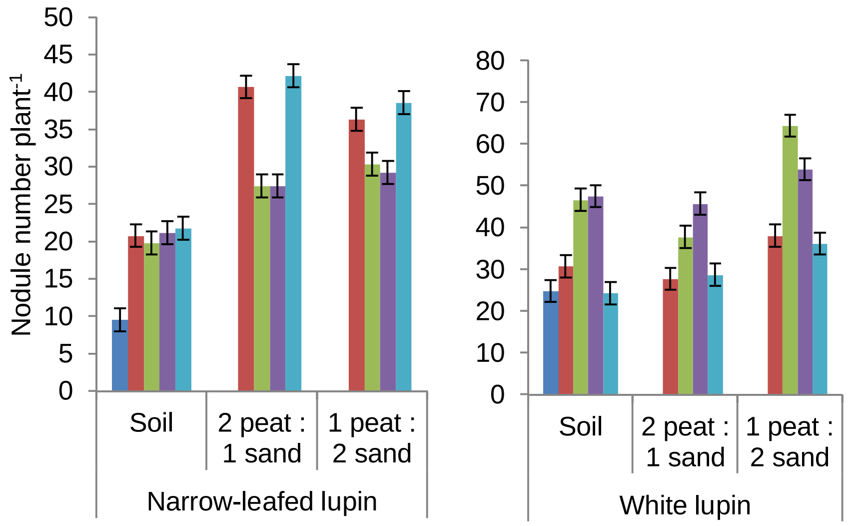

Nodule number plant-1
50
45
40
35
30
25
20
15
10
5
0
80
70
60
50
40
30
20
10
0
Soil
2 peat : 1 sand
1 peat : 2 sand
Narrow-leafed lupin
White lupin

Figure 6. Effects of *Bradyrhizobium* inoculum and potting medium on : (a) shoot dry weight, (b) root dry weight, (c) nodule dry weight, (d) total plant dry weight, (e) plant height and (f) nodule number in narrow-leafed lupin (3 cultivars) and white lupin (1 cultivar) in a greenhouse experiment. Error bars show ± 1 SE. Means of 4 replicates.

3.3 Nitrogen content and biological nitrogen fixation

Species, cultivar and inoculum all had significant effects on shoot nitrogen (%), root nitrogen (%), total plant nitrogen (mg plant^{-1}) and the proportion of the crop nitrogen derived from atmospheric N_2 (% Ndfa) in 2 peat : 1 sand potting medium in the greenhouse experiment (Appendix 5). Total plant nitrogen content and % Ndfa were highest in Ludic and lowest in Sonet (Table 4).

The uninoculated control produced lowest shoot nitrogen (%) , root nitrogen (%) , total plant nitrogen (mg plant^{-1}) and % Ndfa. Shoot and root nitrogen content were increased by 211 and 95% by commercial inoculant, 232 and 144% by HAMBI 3115, 213 and 130% by HAMBI 3116 and 209 and 88% by HAMBI 3118 as compared with uninoculated control. The highest values of total plant nitrogen and biological nitrogen fixation were obtained by using HAMBI 3115 strain (Table 4).

Table 4. Effect of *Bradyrhizobium* inoculation and cultivar on nitrogen concentration, nitrogen accumulation and biological nitrogen fixation in 2 peat : 1 sand potting medium in a greenhouse experiment.

Treatment	Shoot nitrogen (%)	Root nitrogen (%)	Total plant nitrogen (mg N plant^{-1})	% Ndfa
Cultivar				
Haags Blaue	2.95a	1.82b	76c	70.0bc
Sonet	2.92a	1.91b	59c	67.8c
Boruta	2.76a	2.02ab	112b	71.0b
Ludic	2.52b	2.18a	312a	77.0a
SE	0.054	0.061	7.4	0.63
Bradyrhizobium				
Control	1.02b	1.04c	13c	0.0c
Commercial	3.17a	2.04b	165ab	88.2ab
HAMBI 3115	3.39a	2.54a	197a	90.8a
HAMBI 3116	3.20a	2.40a	160b	89.6ab
HAMBI 3118	3.15a	1.96b	162b	87.9b
SE	0.060	0.068	8.3	0.70

The species × inoculum interaction was significant for total plant nitrogen and biological nitrogen fixation. Maximum total plant nitrogen was obtained from white lupin with HAMBI 3115 and the minimum was obtained from narrow-leafed lupin grown in uninoculated control (Figure 7). In narrow-leafed lupin, total plant N decreased in the order HAMBI 3115 > HAMBI 3116 > HAMBI 3118 or its commercial peat inoculant, whereas in white lupin the order was HAMBI 3115 > HAMBI 3118 or its commercial peat inoculant > HAMBI 3116.

In narrow-leafed lupin, atmospheric nitrogen fixation of HAMBI 3116 exceeded that of HAMBI 3118, but the opposite was observed in white lupin. The combination of white lupin and HAMBI 3115 strain showed the greatest % Ndfa, although the differences were small (Figure 8).

There was a significant interaction between *Bradyrhizobium* inoculants and narrow-leafed lupin cultivars on root nitrogen (%). Highest root nitrogen (%) was observed in Sonet with HAMBI 3115 and that of lowest was obtained from Haags Blaue in uninoculated control (Figure 9). Root nitrogen content (% N) of Haags Blaue was almost equal by all three liquid inoculants.

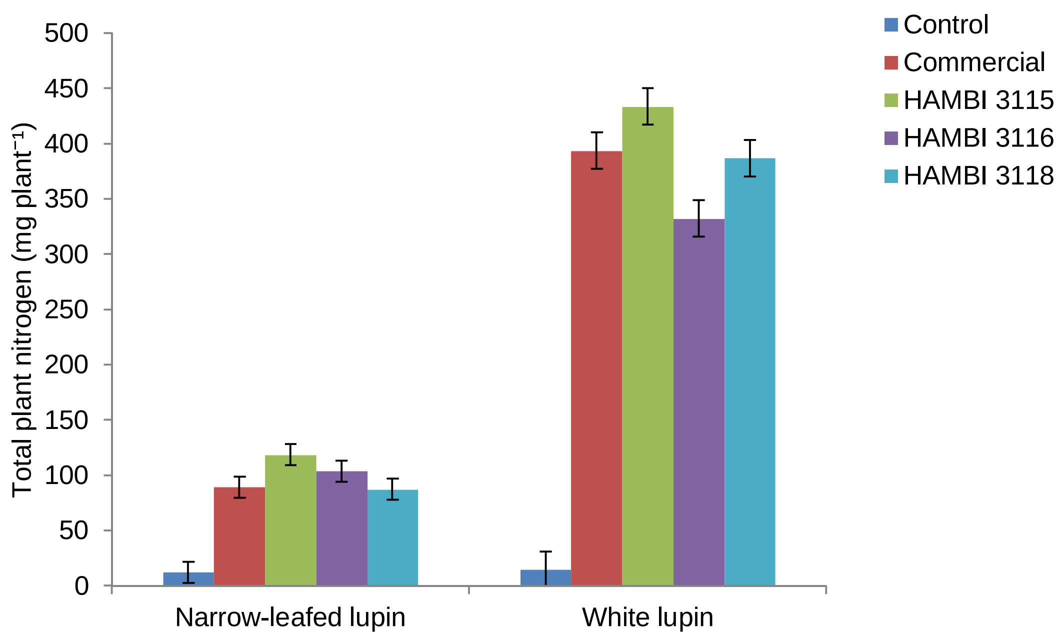


Figure 7. Effects of *Bradyrhizobium* inoculant on total plant nitrogen content in narrow-leafed lupin (3 cultivars) and white lupin (1 cultivar) in a greenhouse experiment. Error bars show ± 1 SE. Means of 4 replicates.

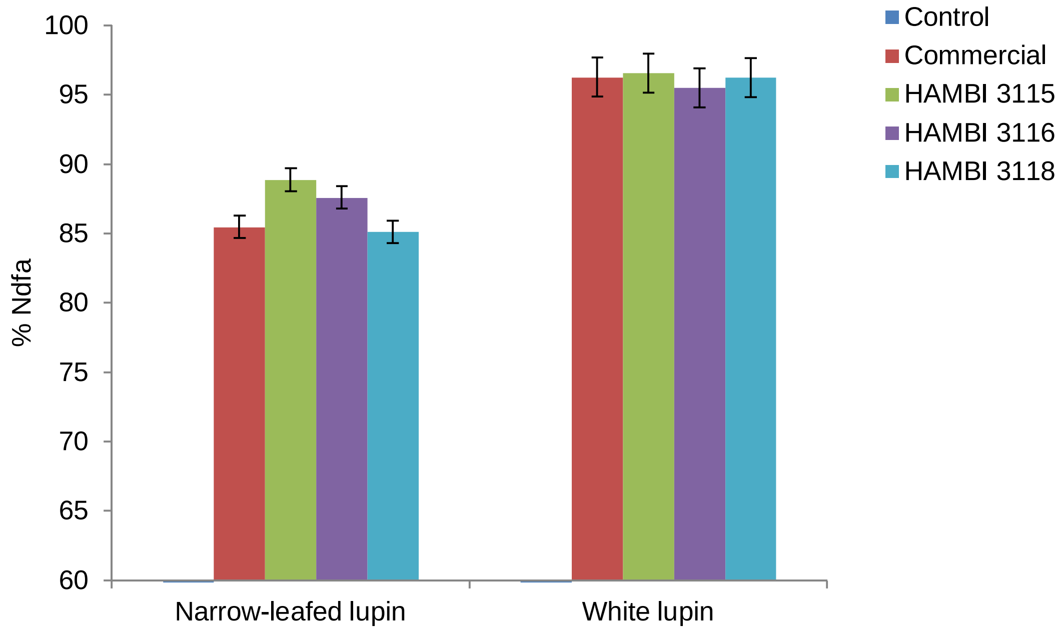


Figure 8. Effects of *Bradyrhizobium* inoculant on biological nitrogen fixation in narrow-leafed lupin (3 cultivars) and white lupin (1 cultivar) in a greenhouse experiment. Error bars show ± 1 SE. Means of 4 replicates.

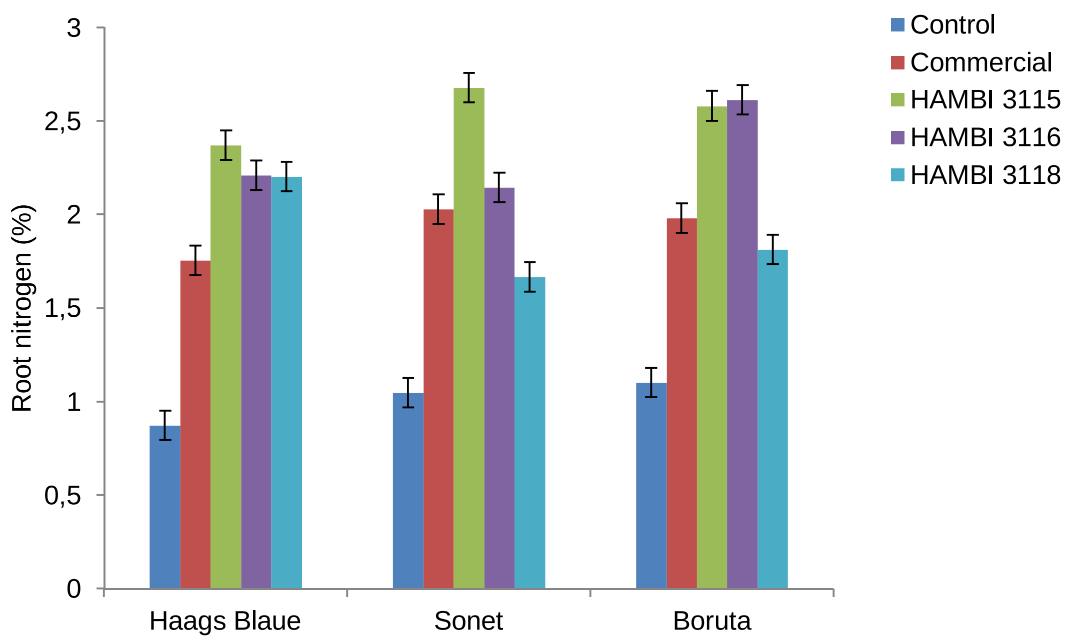


Figure 9. Effects of *Bradyrhizobium* inoculants on root nitrogen content (%) in 3 narrow-leafed lupin cultivars (Haags Blaue, Sonet and Boruta) grown in 2 peat : 1 sand potting medium in a greenhouse experiment. Error bars show ± 1 SE. Means of 4 replicates.

4 DISCUSSION

Significant increase of most of the growth and yield parameters in both lupin species through *Bradyrhizobium* inoculation indicates effective symbiotic association. Among the tested HAMBI strains, HAMBI 3115 performed best on both species. Laboratory HAMBI 3118 and commercial peat-based HAMBI 3118 showed similar results in the greenhouse, but differed in the field, showing the importance of inoculation method and carrier material, and their relationship with field environment. Finnish soil has some nodulating bacteria, but not enough, as shown by the large increase in nodulation and growth following inoculation. Nutrient availability and low pH made Finnish soil more competitive than peat-sand media, which was more obvious in high yielding cultivar than low yielding cultivar.

4.1 Species

Bradyrhizobium inoculants and their performance with narrow-leafed and white lupin cultivars were studied under different potting medium in the controlled environment of a greenhouse experiment. White lupin produced 2-3 fold more shoot, root, nodule and total dry matter compared with the average of three narrow-leafed lupin cultivars. Similar large differences of dry matter accumulations in between these two species were also observed by Clements et al. (1993), Kerley (2000), Römeret et al. (2000), Ayaz et al. (2004a). Interspecific differences in legume dry matter production also depends on growth duration and radiation use efficiency (RUE) (Ayaz et al. 2004b, Ayaz et al. 2004c). The growth duration of white lupin cv. Ludic was longer than those of the three narrow-leafed lupin cultivars, so it intercepted light for a longer time. The shoot and root dry matter accumulations of white lupin cv. Ludic were similar to those of white lupin cv. Lucyanne (Kerley 2000). The dry matter accumulations of the narrow-leafed lupin cultivars were in line with the data of Chen et al. (2011) who found that shoot dry weight ranged between 1.1 and 4.9 g plant^{-1} and root dry weight ranged between 0.4 and 2.4 g plant^{-1}among 10 narrow-leafed lupin accessions.

Plant height, nodule number and chlorophyll index showed positive relationships with dry matter production in both species. Greater plant height in white lupin over narrow-leafed lupin species was also been previously reported (Tang et al. 1993a).

4.2 Cultivar

In both field and greenhouse experiments, cv. Boruta produced considerable more dry matter than cvs Haags Blaue and Sonet, as expected from previous reports (Stoddard et al. 2010) and from its longer growing period, as discussed above.

Shoot, root, nodule and total dry weight accumulation per plant of narrow-leafed lupin cultivars in the greenhouse experiment ranged from 1.79 to 4.04 g $plant^{-1}$, 0.17 to 0.37 g $plant^{-1}$, 0.043 to 0.118 g $plant^{-1}$, and 2.00 to 4.47 g $plant^{-1}$ respectively. Similar ranges of variation were previously reported by many others (Jessop et al. 1990, Clements et al. 1993).

Plant height, root length, nodule number $plant^{-1}$ and chlorophyll index of experimental narrow-leafed lupin cultivars in greenhouse experiment ranged from 47.3 to 51.3 cm, 32.6 to 35.2 cm, 21.9 to 26.0 and 35.1 to 39.8 respectively (Table 4). In Serbia, Mihailovic et al. (2008) observed that plant height of 6 narrow-leafed lupin cultivars ranged between 46 and 57 cm. Similar ranges were also reported for root length (Tang et al. 1993a), nodule number (Tang et al. 1990b, Jessop & Mahoney 1998) and chlorophyll content (Tang et al. 1990a) in narrow-leafed lupin cultivars in Australia.

Plant height, root length and chlorophyll content were lower in the field experiment than in the greenhouse. Dry matter accumulation and seed yield from the field experiment were also lower compared with the results of Dracup et al. (1998), Mihailovic et al. (2008), Annicchiarico & Carroni (2009) (Table 1). Stoddard et al. (2010) also observed higher seed and total dry matter yield of Haags Blaue (3.28 and 7.3 t ha^{-1}) and Boruta (3.94 and 8.8 t ha^{-1}), in an earlier field trial (2009) at Viikki. These indicated a severe reduction of yield and dry matter production in the field condition during the experimental year.

This yield reduction may be associated with the rainless drought condition and high temperature during the growing period, which ultimately shortened the life cycle of the plants and hastened maturity. The highest average temperature was observed on 13-14 July 2010 (Figure 1), during the flowering and early pod filling stage. High temperature stress has been associated with reduced pod number in this species due to abscission of buds and flowers (Downes & Gladstones 1984), leading to reduced yield (French & Turner 1991). Total rainfall accumulation in the growing period of Haags Blaue and Sonet from 26 May to 19 August 2010 was 123.2 mm, while Boruta received an additional 46 mm between 19 and 30 August. Terminal drought can severely reduce dry matter (18-42%), seed yield (95-135%) and harvest index (45-75%) in this crop (Dracup et al. 1998). Reduced plant height and chlorophyll content due to water stress were also observed by Podleśny & Podleśna (2010) in Poland.

The difference in number of seed pod^{-1} among 3 narrow-leafed lupin cultivars was attributable to individual cultivar characteristics as it showed. Higher seed pod^{-1} was observed in Boruta (4.7) than Haags Blaue (3.45) or Sonet (3.44). Results were also similar to the results obtained by Stoddard et al. (2010) who observed higher seed pod^{-1} in Boruta (4.9) than Haags Blaue (3.69). Lower seed pod^{-1} in Sonet (3.7) was also observed by Gresta et al. (2010). Number of pod plant^{-1} observed in narrow-leafed lupin cultivars in field experiment was considerably lower than in the results of Gresta et al. (2010).

4.3 Bradyrhizobium

Bradyrhizobium inoculations significantly increased all the growth and yield components in both greenhouse and field experiments compared to the uninoculated control except root length, in which variation was not significant in both experiment (Appendix 1-4). Significant increase in shoot dry weight (Abd-Alla 1999, Raza et al. 2001, Delić et al. 2010), root dry weight (Abd-Alla 1999, Delić et al. 2010), nodule dry weight (Raza et al. 2001, Delić et al. 2010), seed yield (Raza et al. 2001, Tahir et al. 2009), dry matter yield (Tahir et al. 2009), plant height (Delić et al. 2010, Tahir et al. 2009), nodule number (Raza et al. 2001, Delić et al. 2010), pod plant^{-1} (Tahir et al. 2009) and chlorophyll contents (Vollmann 2011, Tajini et al. 2008) of lupins or other legume crops are been previously reported following inoculation.

In the greenhouse experiment, all the three liquid *B.* sp. (lupin) strains (HAMBI 3115, HAMBI 3116 and HAMBI 3118) and commercial inoculant (peat-based inoculant of HAMBI 3118 strain) were found highly effective for both lupin species. Among the three liquid inoculants, HAMBI 3115 consistently provided the best performance, although the differences were significant only in the case of shoot dry weight, total plant dry weight and plant height in comparison with HAMBI 3118.

The commercial and liquid forms of HAMBI 3118 performed similarly in the greenhouse experiment, but in the field experiment, performance of liquid HAMBI 3118 strain was much lower than its commercial peat form, with significant reduction in shoot dry weight, root dry weight, seed dry weight, total plant dry weight and pod plant^{-1}. Similarly, Kyei-Boahen et al. (2002) observed a significant decrease in total nodule and plant dry weight in desi and kabuli chickpea in field experiments, at

Saskatchewan, Canada, by applying liquid form compared with its peat based inoculant.

Field conditions represent a hostile environment for inoculant cells. High temperature and water deficit reduce bradyrhizobial growth and survival in soil (van Veen et al. 1997, Hungria & Vargas 2000) while also severely reducing the yield and life cycle of the plant. Water deficit also reduces nitrogen fixation (Hungria & Vargas 2000). Use of the right carrier material in inoculant formulation can reduce the bacterial death in adverse soil condition by providing a protective surface, moisture or nutrition (van Veen et al. 1997).

Commercial inoculant was prepared with a peat-based carrier material that is recognized and accepted for inoculants (Date 2001). Feng et al. (2002) also observed better adaptive, survival and stress resistance capacity of *B.* sp. (lupin) inoculated in peat compared with that in broth media.

Nodulation pattern also depends on the inoculation method. Usually after application, bradyrhizobia remain relatively immobile in soil, so seed inoculation results predominantly in crown and tap root nodulation whereas soil inoculation results in nodulation on most parts of the root system (Hardarson et al. 1989, Hardarson & Atkins 2003). The commercial inoculant was mixed with seeds before sowing (26 May), so it remained at the seed depth (2.5-3 cm) and rapidly benefited from rainfall on 26, 29 and 30 May, gaining more opportunity to spread downwards, and making easy and early contact with roots. Lack of bradyrhizobial establishment in the rhizosphere may cause reduction or failure in nodulation (Brockwell & Bottomley 1995). Inoculant placement had a significant influence on nodule numbers and their distribution in roots, with deep placement ensuring better contact with lateral roots and giving significant increases in plant and nodule dry weight (Kyei-Boahen et al. 2002).

Liquid inoculants were applied on the soil surface only after diluting with water, so they remained on the soil surface. Inoculants were sprayed on to the soil surface on 3 June 2010, which turned out to be the middle of an 11-day-long rainless period from 31 May to 10 June (Figure 1). Such dry conditions can cause desiccation and death of bacteria in soil (Kyei-Boahen et al. 2002). Moreover, the overall rainfall during the rest of June and July was also very low, totaling 28.3 and 33.0 mm respectively. These environmental conditions might not be considered as good, as it is well established that the movement of *Bradyrhizobium* in soil is limited and its distribution depends on infiltrating water (Madsen & Alexander 1982). Nevertheless, nodulation was considerably better than in the non-inoculated plots, so while conditions were not ideal, the treatment was effective.

In the greenhouse experiment, however, irrigation was automatic with a supply of 100 ml water pot^{-1} day^{-1}. Regular flushing of the potting medium influences the distribution of *Bradyrhizobium* in the entire pot, ensuring maximum root contact and better nodulation from both inoculation methods. This is confirmed by the often remarkable similarity of the results of the commercial and laboratory samples of HAMBI 3118 in the greenhouse experiment.

4.4 Potting medium

The significant differences in all the growth and yield components among the 3 potting media in the greenhouse experiment revealed the importance of soil type or potting medium in lupin growth.

Growth and yield of lupin plants depend on soil characteristics, pH and nutrient status (Tang & Robson 1993, Tang & Thomson 1996, Kerley 2000, French 2002, Annicchiarico & Alami 2012). Lupin growth is favoured by acidic soil and sensitive to higher pH. Soils containing low lime can increase plant height, shoot, root and nodule dry weight of narrow-leafed lupin over those observed in high lime (Jessop et al. 1990). Higher pH (pH $\geq$ 6) can also decrease nodule number, nodule mass and nitrogen content in both *L. angustifolius* and *L. pilosus* (Tang & Robson 1993).

The soil produced significantly higher values in all measured parameters of lupin growth than the peat-sand media, particularly in white lupin. The reason may be the suitability of the soil for lupin growth and development, as it was acidic (pH 5.8) and came from a field that contained plenty of macro and micro nutrients (Mäkelä-Kurtto & Sippola 2002). Although fertilized peat can also supply good plant nutrition, its bulk density when mixed with sand and other chemical and physical attributes clearly made it less suitable than soil.

Bradyrhizobium was the only source that was responsible for nodulation and nitrogen fixation in peat-sand potting medium, as the observed control plants of peat-sand potting media were entirely nodule free. Soil may contained several non-rhizobium species belonging to α including *Methylobacterium, Blastobacter, Devosia, Phyllobacterium, Ochrobactrum,* and *Agrobacterium,* β including *Cupriavidus, Herbaspirillum,* and *Burkholderia* and γ including *Enterobacter,* and *Pseudomonas* sub-group of proteobacteria that also form nodules and fix nitrogen in legume roots (Benhizia et al. 2004, Balachandar et al. 2007). Trujillo et al. (2005) also observed effective nodulation when white lupin was inoculated with a new species of the genus *Ochrobactrum*. The occurrence of nodules in uninoculated control treatments of Finnish soil indicates the likely presence of indigenous bradyrhizobial species or non-rhizobial species that may also enhance nodulation and nitrogen fixation of plants grown in soil.

4.5 Interactions

The potting medium-inoculation interactions are largely attributable to the presence of some nodulating bacteria in the soil but none in the peat-sand mixes. Responses to inoculation were also greater in the soil than in the peat-sand mixes for shoot dry matter and hence total plant dry matter. Raza et al. (2001b) also stated that the performance of *B.* sp. (lupin) strains greatly depends on soil properties including salinity and soil pH.

The occurrence of species and potting medium interaction in all the parameters of the greenhouse experiment again provided evidence that lupin species differed in their soil preferences. White lupin responded much more strongly to the soil than narrow-leafed lupin, where white lupin shoot mass and root mass in soil were nearly twice those of plants in peat-sand mixes, whereas the corresponding increase in narrow-leafed lupin was only 25%. Significant interaction by species and soil type was also observed by Tang & Robson (1993) and Tang et al. (1993a). Both narrow-leafed and white lupin performed better in soil but the differences in performances were more obvious in white lupin in terms of dry matter accumulation, plant height and chlorophyll content. Similarly, Boruta narrow-leafed lupin was more responsive to soil than the other two cultivars.

Other narrow-leafed lupin cultivars were found to be soil dependent (Jessop et al. 1990). Cultivars having higher yielding capacity require more nutrients for their growth and development and are affected more by different soil types than low-yielding, less nutrient demanding cultivars. Dry matter accumulation and yield of lupin cultivars also depended on soil pH. Soil having pH above 6.0 markedly reduced the nodulation as well as shoot and root dry matter accumulation (Tang & Robson 1993).

The significant interaction between inoculum × lupin species in all the measured parameters in greenhouse experiment indicated that the performance of *Bradyrhizobium* depends on its host species (Abd-Alla 1999). Some species perform better with some specific strains then the others. In terms of growth and yield parameters (plant height and dry matter accumulation) performance order of *Bradyrhizobium* strains in narrow-leafed lupin was HAMBI 3115 > HAMBI 3116 > HAMBI 3118 (or its commercial form) > control and in white lupin was HAMBI 3115 > HAMBI 3118 (or its commercial form) > HAMBI 3116 > control. This interaction was further modified by potting medium, with differences between inoculated and uninoculated being enhanced in white lupin in peat-sand mixtures and reduced in narrow-leafed lupin in soil. The pattern of superiority of HAMBI 3115 in almost all measures was presented, as was the alternation of second rank between HAMBI 3116 for narrow-leafed and 3118 for white lupin.

A significant interactive effect among species, potting medium and *Bradyrhizobium* in shoot, root, nodule and total plant dry weight, plant height and nodule number showed that performance of *Bradyrhizobium* strains depends on different potting medium and species. Nodulation capacity of *Lupinus* species markedly differed with bradyrhizobial strains and soil properties (Abd-Alla 1999). The greater variations in plant height and dry matter accumulations in white lupin with different *Bradyrhizobium* inoculation and potting medium indicated that white lupin species affected more with *Bradyrhizobium* and soil differences compared with narrow-leafed lupin.

4.6 Nitrogen content and biological nitrogen fixation

Shoot nitrogen content of lupin cultivars during pod filling stage was 2.51-2.94%, which was higher than root nitrogen content (1.82-2.17%). These results are in line with those of other leguminous crops (Schulz et al. 1999). Total nitrogen content of the cultivars depended on their dry matter partitioning and relative nitrogen content (%) (Abd-Alla 1999, Delić et al. 2010). *Bradyrhizobium* inoculation increased nitrogen content and biological nitrogen fixation in legume crops (Wadisirisuk et al. 1989, Delić et al. 2010). Plants inoculated with HAMBI 3115 strain performed better than those with HAMBI 3116 and HAMBI 3118 or its commercial form in terms of nitrogen accumulation and biological nitrogen fixation. Compared with non-inoculated control, shoot and root nitrogen content were increased in 232 and 144% by HAMBI 3115, 213 and 130% by HAMBI 3116, 209 and 88% by HAMBI 3118 and 211 and 95% by commercial peat inoculant.

The high (87.9-90.7%) rate of biological nitrogen fixation in experimental lupin cultivars were supported by those of Carranca et al. (2009) who also observed a similar rate (91%) of biological nitrogen fixation in white lupin. All these results showed that experimental strains were highly effective for lupin crops in terms of symbiotic association and biological nitrogen fixation.

5 CONCLUSIONS

The use of *B.* sp. (lupin) inoculants and their interaction with host plants are considered as most important factors for increasing the symbiotic performance in lupin cultivation. Lupin cultivars also have specific soil preference which may affect *Bradyrhizobium*-host compatibility.

In both field and greenhouse conditions significant increases in lupin growth and yield parameters occurred following *B.* sp. (lupin) inoculation. HAMBI 3115 strain showed better results compared with other two liquid strains HAMBI 3116 and HAMBI 3118. Performance of lupin cultivars also varied with different potting media in the greenhouse experiment where soil showed better results than the two peat-sand mixes. Inoculation with HAMBI 3115 in soil can be considered as best combination for lupin as it showed highest growth, yield, nodules, chlorophyll index, nitrogen content (% N) and biological nitrogen fixation in the greenhouse experiment and the best of three liquid inoculants in the field experiment. HAMBI 3115 can be used for peat-based inoculant for its superiority over HAMBI 3118.

Further studies are needed to understand the ecology and durability of *Bradyrhizobium* strains in soil as well as inoculant types, inoculation methods, use of carrier materials and their effect on strain survival in adverse field conditions. Research can also be done to evaluate the performance of *Bradyrhizobium* strains in single inoculation or mixed inoculation.

6 ACKNOWLEDGEMENTS

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8 APPENDICES

Appendix 1: Summary of the analysis of variance (mean square values) of shoot dry weight, root dry weight, seed dry weight, total plant dry weight and harvest index of narrow-leafed lupin (3 cultivars) in a field experiment at harvest as affected by *Bradyrhizobium* inoculation and cultivar.

Treatment	df	Mean square				
		Shoot dry weight ($g\ m^{-2}$)	Root dry weight ($g\ m^{-2}$)	Seed dry weight ($g\ m^{-2}$)	Total dry weight ($g\ m^{-2}$)	Harvest index (%)
Bradyrhizobium	4	7926***	53.7***	10833**	39272***	34.116***
Main plot residual	15	478	6.3	423	1816	2.965
Cultivar	2	17996***	157.3***	4413***	44108***	60.465***
Bradyrhizobium * cultivar	8	429	1.1	316	1460	0.931
Sub plot residual	30	565	7.5	233	1564	2.688

*, **, *** = significant at P < 0.05, P < 0.01, P < 0.001, respectively.

Appendix 2: Summary of the analysis of variance (mean square values) of plant height, root length, seed pod^{-1}, pod plant^{-1} and chlorophyll index of narrow-leafed lupin (3 cultivar) in a field experiment as affected by *Bradyrhizobium* inoculation and cultivar.

Treatment	df	Mean square				
		Plant height (cm)	Root length (cm)	Seed pod^{-1}	Pod plant^{-1}	Chlorophyll index (SPAD unit)
Bradyrhizobium	4	21.06*	0.476	0.007	5.907***	51.8*
Main plot residual	15	10.54	0.8	0.011	0.3161	13.9
Cultivar	2	60.32***	6.29***	11.247***	0.4700	41.2
Bradyrhizobium * cultivar	8	1.07	0.033	0.0013	0.1637	1.4
Sub plot residual	30	5.02	0.238	0.003	0.182	14.3

*, **, *** = significant at P < 0.05, P < 0.01, P < 0.001, respectively.

Appendix 3: Summary of the analysis of variance (mean square values) of shoot dry weight, root dry weight, nodule dry weight and total plant dry weight of narrow-leafed (3 cultivars) and white lupin (1 cultivar) in a greenhouse experiment as affected by species, cultivar, potting medium and *Bradyrhizobium*.

Source	df	Mean square			
		Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Nodule dry weight (g plant^{-1})	Plant dry weight (g plant^{-1})
Species	1	4603.68***	23.544***	5.251***	5624.114***
Cultivar	2	85.99***	0.317***	0.088***	100.188***
Potting medium	2	304.66***	0.092***	0.157***	329.093***
Bradyrhizobium	4	104.95***	0.270***	0.172***	124.785***
Potting medium * *Bradyrhizobium*	8	4.54***	0.025***	0.013***	5.721***
Species * Potting medium	2	363.71***	0.437***	0.228***	408.404***
Cultivar * Potting medium	4	13.13***	0.054***	0.005**	14.981***
Species * *Bradyrhizobium*	4	107.40***	0.507***	0.146***	131.156***
Cultivar * *Bradyrhizobium*	8	1.54	0.001	0.002	1.647
Species * Potting medium * *Bradyrhizobium*	8	2.84*	0.066***	0.006***	3.987**
Cultivar * Potting medium * *Bradyrhizobium*	16	0.08	0.001	0.000	0.087
Error	180	1.28	0.006	0.001	1.481

*, **, *** = significant at P < 0.05, P < 0.01, P < 0.001, respectively.

Appendix 4: Summary of the analysis of variance (mean square values) of plant height, root length, nodule number and chlorophyll index of narrow-leafed (3 cultivars) and white lupin (1 cultivar) in a in a greenhouse experiment as affected by species, cultivar, potting medium and *Bradyrhizobium*.

Source	df	Mean square			
		Plant height (cm)	Root length (cm)	Nodule number	Chlorophyll index (SPAD unit)
Species	1	56831.48***	853.06***	3952.45***	3574.20***
Cultivar	2	246.59***	329.32***	280.73***	335.43***
Potting medium	2	1596.62***	531.74***	1073.45***	1068.31***
Bradyrhizobium	4	3174.98***	10.33	7443.51***	1068.08***
Potting medium * *Bradyrhizobium*	8	395.47***	12.28	786.41	90.38
Species * Potting medium	2	572.02***	189.09***	984.39***	205.13***
Cultivar * Potting medium	4	2.95	29.67	295.00***	15.85
Species * *Bradyrhizobium*	4	1151.29***	105.49**	1575.38***	108.64**
Cultivar * *Bradyrhizobium*	8	7.25	11.35	29.78	17.04
Species * Potting medium * *Bradyrhizobium*	8	186.87***	34.97	87.56**	13.28
Cultivar * Potting medium * *Bradyrhizobium*	16	2.56	12.14	39.97	2.03
Error	180	25.73	23.65	28.32	26.99

*, **, *** = significant at P < 0.05, P < 0.01, P < 0.001, respectively.

Appendix 5: Summary of the analysis of variance (mean square values) of shoot nitrogen, root nitrogen, total plant nitrogen and biological nitrogen fixation of narrow-leafed (3 cultivars) and white lupin (1 cultivar) that grown in 2:1, peat and sand in a in a greenhouse experiment as affected by species, cultivar and *Bradyrhizobium*.

Treatment	df	Mean square			
		Shoot nitrogen (%)	Root nitrogen (%)	Total plant nitrogen (mg plant^{-1})	% Ndfa
Species	1	1.921***	0.844**	792773***	845.24***
Cultivar	2	0.02*	0.101	14238***	42.72**
Bradyrhizobium	4	15.728***	5.496***	84193***	25432.71***
Species * *Bradyrhizobium*	4	0.098	0.095	52094***	60.36***
Cultivar * *Bradyrhizobium*	8	0.073	0.177*	954	4.50
Error	60	0.058	0.075	1092	7.93

*, **, *** = significant at P < 0.05, P < 0.01, P < 0.001, respectively.